PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

DICHLOROMETHANE (METHYLENE CHLORIDE, DCM)

September 2000

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Public Health Goal for
DICHLOROMETHANE (METHYLENE CHLORIDE, DCM)
In Drinking Water

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PREFACE

Drinking Water Public Health Goals

Pesticide and Environmental Toxicology Section

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based upon currently available data and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs adopted by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR DICHLOROMETHANE IN DRINKING WATER

SUMMARY

A Public Health Goal (PHG) of 0.004 milligrams per liter (mg/L) or 4 micrograms per liter (μg/L) or 4 parts per billion (ppb) has been developed for dichloromethane (DCM), also known as methylene chloride, in drinking water. The PHG is based on carcinogenic effects observed in experimental animals. Carcinogenicity of DCM has been observed in 1) female rats and male mice in a two-year drinking water study sponsored by the National Coffee Association (Serota et al. 1986a, 1986b; National Coffee Association 1982, 1983), and 2) male and female rats and male and female mice in a two-year inhalation study by the National Toxicology Program (NTP 1986, Mennear et al. 1988). DCM administered in the drinking water induced a significant increase in combined hepatocellular carcinoma and neoplastic nodules in female Fischer 344 rats and in male B6C3F1 mice (Serota et al. 1986a, 1986b). The inhalation studies with DCM showed an increased incidence of liver and lung tumors in both male and female B6C3F1 mice (NTP 1986). The NTP (1986) inhalation study and the Serota et al. (1986b) drinking water ingestion study cited in this document for the development of the PHG provided evidence for the carcinogenicity of DCM in rodents. The evidence is supported by additional findings in at least five other animal carcinogenicity studies and at least ten human epidemiological studies. It is further supported by findings of genotoxicity in bacterial and mammalian systems both *in vitro* and *in vivo*. Office of Environmental Health Hazard Assessment (OEHHA) scientists evaluated all available studies and the results indicate that DCM is an animal carcinogen in at least two animal species, in male and female animals, and the tumors occur at multiple sites.

For the calculation of the PHG, cancer potency estimates were based on the recommended practices of the 1996 United States Environmental Protection Agency (U.S. EPA) proposed guidelines for carcinogenic risk assessment (U.S. EPA 1996b), in which the polynomial [similar to that used in the linearized multistage (LMS) model, but used without low dose extrapolation] is fit to the experimental data in order to establish the lower 95 percent confidence bound on the dose associated with a 10 percent increased risk of cancer (LED_{10}). The physiologically based pharmacokinetic (PBPK) model used by the Occupational Safety and Health Administration (OSHA) in its final rules on occupational exposure to DCM (OSHA 1997) was applied with both prior and posterior mean parameters used in a Bayesian analysis of parameter uncertainty. The PBPK model was adjusted to allow oral as well as inhalation exposure and the dose metric employed was the daily mass of glutathione sulfotransferase (GST) metabolites generated by the target tissues in the liver and lung expressed on a body weight basis (mg GST metabolites/kg-day). Based on the prior parameters, the cancer slope factor (CSF) of the combined male mouse liver adenomas and carcinomas in the oral study (Serota et al. 1986b) was 5.7×10^{-3} (mg/kg-day)$^{-1}$, and the geometric mean of the CSFs from male and female mouse liver and lung tumors in the inhalation study (NTP 1986) was 1.7×10^{-3} (mg/kg-day)$^{-1}$. Based on the posterior parameters, the CSF of the combined male mouse liver adenomas and carcinomas in the oral study (Serota et al. 1986b) was 4×10^{-3} (mg/kg-day)$^{-1}$, and the geometric mean of the CSFs from male and female mouse liver and lung tumors in the inhalation study (NTP 1986) was 2.4×10^{-3} (mg/kg-day)$^{-1}$.

The PHG was calculated assuming a *de minimis* theoretical excess individual cancer risk level of one in a million (10^{-6}) from exposure to DCM. Using the above-mentioned four CSFs with daily water consumption (DWC) rates of 2.2 liters per day (L/day) from direct drinking water consumption and 3.8 liters equivalent per day (L_{eq}/day) from inhalation and dermal exposure to DCM volatilized from drinking water (Bogen et al. 1992), concentrations of 3.7 ppb and 3.9 ppb were derived based on the prior mean and posterior mean CSFs, respectively. OEHHA rounded these values to 0.004 mg/L or 4 ppb for the DCM drinking water PHG. The adopted PHG is considered to contain an adequate margin of safety for the potential noncarcinogenic effects, including adverse effects on the pulmonary, hepatic, renal, cardiac, hematopoietic, neurological, endocrine, and reproductive and developmental systems.

In addition to the value of 4 ppb based on carcinogenicity, a health protective value of 0.133 mg/L or 133 ppb was calculated based on noncancer effects of liver toxicity in male and female rats from the Serota et al. (1986a) two-year drinking water study. The liver effect is the most sensitive noncarcinogenic effect by the oral route observed in experimental animals with a no observable adverse effect level (NOAEL) of 6 mg/kg-day. This value of 133 ppb incorporates two 10-fold uncertainty factors (UFs), one each for interspecies and interindividual variation. The 133 ppb value also incorporates a DWC rate of 6 L_{eq}/day as the sum of 2.2 L/day from direct drinking water consumption and 3.8 L_{eq}/day from inhalation and dermal exposure to DCM volatilized from drinking water. In addition, the 133 ppb value incorporates a relative source contribution (RSC) default value of 20 percent. The default value for water ingestion is the same as used by U.S. EPA Office of Water, and is also documented in OEHHA's draft technical support document "Exposure Assessment and Stochastic Analysis" (OEHHA 1996). The use of a 20 percent RSC indicates that most of the DCM exposure occurs from ambient air levels. It is used in the noncancer risk assessment, but, consistent with standard practice, is not incorporated into the cancer risk assessment. The 133 ppb value does not protect the public against the potential carcinogenicity of DCM in humans. Therefore the lower value of 4 ppb is adopted as the PHG since it is based on a quantitative assessment and is thought to have less uncertainty associated with it than the higher value of 133 ppb.

The U.S. EPA (1992b) has promulgated a Maximum Contaminant Level (MCL), which is the enforceable primary drinking water standard, of 5 ppb and a Maximum Contaminant Level Goal (MCLG) of zero for DCM. The U.S. EPA (1992b, 1999) used the NTP (1986) inhalation study and the Serota et al. (1986a, 1986b) oral studies to derive the MCL in 1992. California adopted the federal MCL of 5 ppb as the State MCL for DCM in 1994.

INTRODUCTION

The purpose of this document is to establish the basis for a PHG for DCM in drinking water. DCM is a synthetic volatile chemical without known natural sources. The production of DCM exceeds 200 million pounds annually in the United States (U.S.) and it is used in more than ten industries. In 1993, the U.S. EPA characterized DCM as the third most commonly used halogenated solvent based on 1991 data. DCM is used principally as a solvent in the removal of paint and grease; as a propellant for aerosolizing paints, automotive products, and insect sprays; and as a process solvent in the manufacture of foam polymers, pharmaceuticals and photographic film coatings. Other major uses are as a cleaner in electronic manufacturing, a degreening agent for citrus fruits, and a blowing agent in the production of urethane foam. Sources of DCM

contamination in drinking water are mainly discharges from pharmaceutical and chemical factories. DCM has been detected in finished and raw water supplies in the U.S., and in

groundwater wells used as drinking water sources in California at median concentrations ranging from 0.7 to 10 μg/L (ppb).

The animal and human evidence summarized in this report indicates that DCM has the potential to cause cancer, cardiac effects, central nervous system (CNS) damage, liver toxicity and other health effects. DCM is classified by the U.S. EPA (1984, 1985a, 1985b, 1992a, 1999) as a Group B2 probable human carcinogen and by the International Agency for Research on Cancer (IARC 1986, 1987, 1999) of the World Health Organization (WHO) in Group 2B as possibly carcinogenic to humans. The European Community (EC) classifies and labels DCM as Xn harmful, R20 harmful through inhalation exposure, and a Category 3 Carcinogen with R40 possible risk of irreversible effects as summarized by the Royal Society of Chemistry (RSC), United Kingdom (RSC 1986). The National Toxicology Program (NTP 1986) classifies DCM as a class 2 carcinogen; a chemical that may reasonably be anticipated to be a carcinogen. The Occupational Safety and Health Administration (OSHA 1997) concludes that DCM is a potential occupational carcinogen. The American Conference of Governmental Industrial Hygienists (ACGIH 1986, 1990, 1995) classifies DCM as an A2 suspected human carcinogen, and the U.S. National Institute for Occupational Safety and Health (NIOSH 1976, 1992a, 1992b, 1993) classifies DCM as a potential occupational carcinogen. The U.S. Consumer Product Safety Commission (CPSC 1986, 1987, 1990) and the U.S. Food and Drug Administration (FDA 1989) also conclude that DCM is an animal carcinogen and may be carcinogenic to humans.

The Agency for Toxic Substances and Disease Registry (ATSDR) of U.S. Centers for Disease Control and Prevention (CDC) published and updated toxicological profiles for DCM (ATSDR 1987, 1989, 1993). The EC Organization for Economic Cooperation and Development (OECD 1994) published an evaluation of means for DCM risk reduction as a part of a series of monographs to provide experience with reducing DCM risk in EC countries. The International Programme on Chemical Safety (IPCS), a joint venture of the United Nations Environmental Programs, the International Labour Organization, and the WHO, published Environmental Health Criteria on DCM in 1984 and later updated it as the second edition in 1996 (IPCS 1996). The Canadian government drafted in 1987 a Priority Substances List Assessment Report on DCM to assess the toxicity of DCM and later finalized it in 1993 (Environment Canada 1993, Long et al. 1994).

After evaluating DCM for about 10 years, the OSHA (1997) determined that DCM poses significant risks for workers at the previous exposure standard of 500 ppm eight-hour time-weighted average (TWA) which was adopted in 1971. In its final rule on DCM, OSHA provided quantitative risk assessment using PBPK models and established an occupational exposure limit of 25 ppm for an eight-hour workday and a short-term exposure limit of 125 ppm for 15-minute durations for persons who work with DCM. NIOSH recommends a permissible limit of 75 ppm of DCM over a 10-hour workday in the presence of carbon monoxide (CO) concentrations less than or equal to 9.9 ppm.

The U.S. EPA requires that annual releases of DCM of 1,000 pounds or more be reported to the federal government. The U.S. EPA (1992b) has promulgated a Maximum Contaminant Level (MCL), which is the enforceable primary drinking water standard, of 5 μg/L or 5 ppb and a Maximum Contaminant Level Goal (MCLG) of zero for DCM. California also has a MCL of 5 ppb for DCM. The U.S. EPA Health Advisory recommends that exposure of a 10-kg child to DCM in drinking water should not exceed 10 ppm (mg/L) for one day, or 2 ppm for 10 days. To establish the MCL, the U.S. EPA (1992b, 1999) used the NTP (1986) inhalation study and the

Serota et al. (1986a, 1986b) oral studies to derive the MCL of 5 ppb for DCM in 1992. California

adopted the federal MCL of 5 ppb as the State MCL for DCM in 1994 (CCR Title 22, Article 5.5, Section 64444, Table 64444-A).

California Air Resources Board (ARB 1989) listed DCM as a Toxic Air Contaminant (TAC) under Chapter 1047, Statutes of 1983, in its July 1989 Board hearing. OEHHA, previously a part of the California Department of Health Services (DHS) before the formation of California Environmental Protection Agency (Cal/EPA) in 1991, listed DCM as a Proposition 65 carcinogen. In 1989, OEHHA scientists from the Air Toxicology and Epidemiology Section evaluated DCM for the ARB to provide part of the technical support to list DCM (DHS 1989). This PHG document is an update of that earlier health risk assessment of DCM. The major portion of this evaluation is based on the U.S. EPA (1984, 1985a, 1985b, 1987a, 1987b, 1992a, 1992b, 1994a, 1994b, 1994c, 1999) health assessment documents for DCM, the ATSDR (1987, 1989, 1993) toxicological profiles for DCM, OSHA (1997) final rules on DCM, and documents from OECD (1994) and IPCS (1996) with additional updated literature. The information on exposure assessment contained in this report is adapted from Bogen et al. (1992) and DHS (1989).

The purpose of this review is to develop a PHG for DCM in drinking water based on a comprehensive analysis of the information on the toxicology of DCM. PHGs are based solely on health effects without regard to cost impacts or other factors and, for carcinogens, are set at one in a million (10^{-6}) risk level. Since the absorption and elimination kinetics of DCM were consistent with results generated by both inhalation and oral exposures, cancer potency values used in the determination of the PHG were developed based on the modified physiologically based pharmacokinetics (PBPK) model approach according to the OSHA (1997) final rules. Using the prior and posterior mean parameters, the cancer slope factor (CSF) was derived based on the combined male mouse liver adenomas and carcinomas in the oral study (Serota et al. 1986b) and the geometric mean of the CSFs from male and female mouse liver tumors in the inhalation study (NTP 1986). Based on a standard default human adult body weight of 70 kg and an estimated daily water consumption (DWC) rate of 2.2 liters per day (L/day) from ingestion with 3.8 liters equivalent per day (L_{eq}/day) from inhalation and dermal exposures, the calculated health-protective concentrations were 3.7 μg/L and 3.9 μg/L using the CSFs from the prior and the posterior mean parameters, respectively. The PHG has been set at 4 μg/L, or 4 ppb, rounded from the average of these numbers.

CHEMICAL PROFILE

Chemical Identity

DCM [CH_2Cl_2, CAS Registry Number 75-09-2] is a synthetic chemical without known natural sources (RSC 1986). The chemical structure, synonyms, and identification numbers are listed in Table 1 and are adapted from the Merck Index (Merck 1989), the Environmental Defense Fund (EDF) Chemical Scorecard computerized database (EDF 1999), the Hazardous Substances Data Bank (HSDB) of the National Library of Medicine (HSDB 1999), the Integrated Risk Information System (IRIS) of U.S. EPA (1999), TOMES (Toxicology and Occupational Medicine System) PLUS® (Hall and Rumack 1999) computerized database, University of Utah (1998) Material Safety Data Sheet computerized database, and the ATSDR (1993), IPCS (1996), OECD (1994), RSC (1986), and U.S. EPA (1984, 1985a, 1985b, 1994b) documents.

For DCM, TOMES PLUS® computerized database (Hall and Rumack 1999) contains entries in HAZARDTEXT®, MEDITEXT®, REPROTEXT® System, REPROTOX® System of Georgetown University, Shepard's Catalog of Teratogenic Agents of the Johns Hopkins University, HSDB, IRIS, Registry of Toxic Effects of Chemical Substances (RTECS®) of NIOSH, Chemical Hazard Response Information System (CHRIS) of U.S. Coast Guard, Oil and Hazardous Materials/Technical Assistance Data System (OHM/TADS) of U.S. EPA, Department of Transportation (DOT) Emergency Response Guide, New Jersey Hazardous Substance Fact Sheets (NJHSFS), North American Emergency Response Guidebook Documents (NAERG) of U.S. DOT, Transport Canada and the Secretariat of Communications and Transportation of Mexico, and the NIOSH Pocket Guide$^{(TM)}$.

Physical and Chemical Properties

Important physical and chemical properties of DCM, given in Table 2, are from Merck Index (1989), together with the HSDB (1999), RTECS® (1999), TOMES PLUS® (Hall and Rumack 1999), and University of Utah (1998) Material Safety Data Sheet computerized database, and the ATSDR (1993), Environment Canada (1993), OECD (1994), IPCS (1996), and U.S. EPA (1985a, 1985b, 1994b) documents. Detailed physical and chemical properties have been reported by the RSC (1986).

DCM is a halogenated aliphatic hydrocarbon with a molecular weight of 84.93 g/mole, a boiling point of 40°C (also cited as 39.8°C) or 104°F at 760 mm Hg, a specific gravity of 1.3, a vapor density of 2.9 and a vapor pressure of 350 mm Hg at 20°C or 68°F. The concentration of DCM in saturated air at 25°C reaches 550,000 ppm. DCM has a lower explosive limit of 12 percent and an upper explosive limit of 19 percent and therefore is used as a flame suppressant in solvent mixtures. DCM is a volatile organic compound (VOC) with a penetrating sweetish odor resembling ether or chloroform. It is a clear, colorless liquid at room temperature. The odor threshold for DCM varies between 100 and 300 ppm (Alexander et al. 1982, OSHA 1997). The odor threshold is estimated to be approximately 200 or 250 ppm in air and approximately 9 ppm in water (Amoore and Hautala 1983, ATSDR 1993).

DCM is generally nonflammable, corrosive only to some plastics and coatings, and nonexplosive under ordinary conditions. It can be flammable in oxygen, or in air at 102°C or higher temperature. In the presence of water, DCM is stable but can produce corrosive hydrogen chloride. Elevated temperatures at or over 102°C or the presence of alkalis or metals accelerate this reaction. Contact with strong oxidizers, caustics and active metal powder may cause explosions and fires. DCM forms an explosive mixture in an atmosphere with a high oxygen content, or in the presence of liquid oxygen, nitrite, potassium, or sodium. It is combustible when exposed to heat or flame or spark. Most vapors containing DCM are heavier than air. Vapors may form explosive mixtures with air when ignited. When heated to decomposition, it emits toxic fumes of phosgene, hydrogen chloride, carbon monoxide (CO) and carbon dioxide (CO_2). Fire produces similar irritating, corrosive or toxic gases. Runoff from fire control contains DCM and its combustion products (EDF 1999, HSDB 1999, RTECS 1999).

DCM is soluble in water, alcohols, aldehydes, ethers, ketones, phenols, and organic liquids. It is miscible with chlorinated solvents, diethyl ether, and ethanol. It has low water solubility of 13 to 20 grams (g) per 1,000 g of water at 20°C, extensive oil and fat solubility, and a low flammability potential. The octanol-water partition coefficient K_{ow} is reported to be 25 (ATSDR

1993). The log of the octanol-water partition coefficient (log K_{ow}) is also reported to be approximately

1.3 which indicates that there is at least 10 times more partitioning of DCM in lipids than in an aqueous phase (Hansch and Leo 1979, Howard 1990). The molecular size and log K_{ow} of DCM are characteristic of molecules which are able to penetrate across biological membranes of the skin, lungs and gastrointestinal tracts. One part per million (ppm) of DCM, volume to volume in air, is about 3.47 to 3.53 mg/m^3 of air at 25°C (HSDB 1999).

Table 1. Chemical Identity of Dichloromethane (DCM)

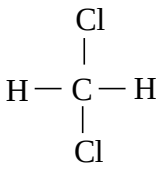
Characteristic	Information	Reference
Chemical Name	Dichloromethane	Merck 1989
Synonyms	Methylene chloride, DCM, MC, methylene dichloride, methylene bichloride, methane dichloride, 1,1-dichloromethane, methylenum chloratum	Merck 1989
Chemical Abstracts Service (CAS) name (9th Collective Index)	Methane, dichloro-	IPCS 1996
IUPAC Systematic Name	Dichloromethane	IARC 1999
Registered trade names	Freon 30, R30, R 30 Narkotill, Aerothene MM, Solaesthin, Solmethine	RTECS 1999
Chemical formula	CH_2Cl_2	Merck 1989
Chemical structure		HSDB 1999

Table 1 (Continued). Chemical Identity of Dichloromethane (DCM)

Characteristic	Information	Reference
Identification numbers:		
CAS registry number	75-09-2	Merck 1989
Beilstein reference number	BRN 1730800	RTECS 1999
Beilstein handbook reference	4-01-00-00035	RTECS 1999
Wiswesser line notation	G1G	RTECS 1999
DOT/United Nations/North America/ International Maritime Dangerous Goods Code (DOT/UN/NA/IMCO) shipping number	IMO 6.1 UN1593	RTECS 1999
HSDB number	66	HSDB 1999
National Cancer Institute (NCI) number	NCI-C50102	RTECS 19
NIOSH RTECS number	PA8050000	RTECS 1999
U.S. EPA RCRA waste number	F002 U080	HSDB 1999
U.S. EPA (OHM/TADS) number	7217234	HSDB 1999
NAERG guideline number	160	TOMES 1999
STCC number	49 411 32	HSDB 1999
European Economic Community (EC) registry index number	602-004-00-3	OECD 1994
EINECS registry number	200-838-9	OECD 1994

Table 2. Chemical and Physical Properties of Dichloromethane

Property	Value or information	Reference
Molecular weight	84.93 g/mole	Vershueren 1983
Color	colorless	Vershueren 1983
Physical state	liquid	Vershueren 1983
Melting point	-95.1°C or -139°F	Weast 1985
Boiling point	40°C or 104°F	Weast 1985
Density at 20°C	1.3266 g/mL	Weast 1985
Solubility		
in water, 20°C	13 - 20 g/L water	OECD 1994
in water, 20°C	1.38 g/100 mL water	IARC 1999
in water, 20°C	20,000 mg/L water	Vershueren 1983
in water, 25°C	16,700 mg/L water	Vershueren 1983
in organic solvents	soluble in alcohol and ether	Vershueren 1983
Partition coefficients		
octanol-water (K_{oc})	25	Roy and Griffin 1985, ATSDR 1993
octanol-water	17.8	Leo et al. 1971
Log K_{ow}	1.3	Hansch and Leo 1979
	1.25	Howard 1990
Log K_{oc}	0.89 (calculated from K_{ow})	IPCS 1996
Vapor pressure		
at 20°C	470/349 mm Hg	Vershueren 1983
at 24.1°C	400 mm Hg	HSDB 1999
at 25°C	436 mm Hg	OECD 1994
Henry's law constant		Mackay and Shiu 1981
at 25°C	2.03×10^{-3} atm-m^3/mole	
at 25°C	2.03×10^{-3} atm-m^3/mole	ATSDR 1993
	2.57×10^{-3} atm-m^3/mole	U.S. EPA 1994b
at 20°C	380 Pa-m^3/mole	IPCS 1996
at 15°C	1.31×10^{-3} atm-m^3/mole	Gossett 1987
Flash point	incombustible	OECD 1994
Ignition temperature	605°C	OECD 1994

Table 2 (Continued). Chemical and Physical Properties of Dichloromethane

Property	Value or information	Reference
Explosion limits (with a high energy source only)	lower 13% (v/v) upper 22% (v/v)	OECD 1994
Odor	Sweetish Ethereal	Vershueren 1983 IPCS 1996
Odor threshold:		
Water	9.1 ppm	Amoore and Hautala 1983
Air	540 - 2,160 mg/m^3	
	(~160 - 620 ppm)	HSDB 1999
	250 ppm	Amoore and Hautala 1983
	883 mg/m^3	
	(~250 ppm)	OECD 1994
	205 - 307 ppm	CHRIS 1999
	214 ppm	U.S. EPA 1994b
Conversion factors		
ppm (v/v) to mg/m^3	1 ppm = 3.53 mg/m^3	Vershueren 1983
in air at 25°C	1 ppm = 3.48 mg/m^3	U.S. EPA 1994b
	1 ppm = 3.47 mg/m^3	OECD 1994
mg/m^3 to ppm (v/v)	1 mg/m^3 = 0.28 ppm	Vershueren 1983
in air at 25°C	1 mg/m^3 = 0.288 ppm	U.S. EPA 1994b

Production and Uses

DCM is mainly produced by a two-stage reaction involving reacting methanol vapor with gaseous hydrogen chloride then with gaseous chlorine. It can also be made by direct chlorination of methane gas. Worldwide production of DCM was estimated to be 570,000 metric tons (tonnes) in 1992 which was about the same as the production in 1980, and the production in 1991 was about 437,000 tonnes (OECD 1994, HSDB 1999). The total amount produced in Western Europe ranged from 331,500 tonnes in 1986 to 254,200 tonnes in 1991. The largest companies producing DCM in Western Europe are located in Great Britain and Germany (OECD 1994). In Canada, DCM has been imported without domestic production and approximately in the range of 9,000 to 13,200 tonnes were used annually during the period from 1977 to 1990 (Environment Canada 1993, Long et al. 1994). The world production of DCM in 1980 was estimated, as cited in another report (NTP 1986, Mennear et al. 1988), to be 517 million kilograms (kg) and the U.S. production was about 270 million kg.

In the U.S., three manufacturers with five plants produce DCM, none of which is located in California. Annual production capacity in the U.S. was about 672 million pounds in 1985 although total production was about 467 million pounds in that year. Nearly 63 million pounds DCM produced in the U.S. in 1985 were exported, and about 56 million pounds were imported. DCM production decreased in 1985 with estimates down about 10 to 15 percent from 1984 (ARB 1989). An estimated 607 million pounds of DCM were manufactured by four domestic producers in 1984, of which about 501 million pounds were sold. Exports in 1984 exceeded 54 million pounds and imports were about 44 million pounds. DCM production by the U.S. chemical industry was reported to be about 745 million pounds in 1977, about 931 million pounds in 1979, about 891 pounds in 1981, about 584 million pounds in 1983, about 559 million pounds in 1986 (Anonymous 1987), and about 500 million pounds or 229,000 tonnes in 1988 (ATSDR 1993, OECD 1994, IPCS 1996). In 1992, about 350 million pounds of DCM were produced and about 13 million pounds were imported (U.S. EPA 1994b).

The production and demand for DCM have continued to decline as more manufacturers move toward water-based aerosol systems in anticipation of further regulation of DCM due to health concerns (ARB 1989, HSDB 1999, NTP 1998). Based on data supplied by the Halogenated Solvents Industry Alliance (HSIA) to the ARB, the amounts of DCM shipped to California by domestic manufacturers ranged from 30,408 tons in 1980 to 23,462 tons in 1985 (ARB 1989).

DCM is mainly used as a paint-stripping and degreasing solvent accounting for about 35 percent of the 1992 U.S. consumption or approximately 125 million pounds. The second largest application, accounting for about 25 percent or approximately 88 million pounds, is in chemical processing for the manufacture of steroids, antibiotics, vitamins, and tablet coatings (U.S. EPA 1994b). In California, paint removers account for the largest use of DCM with an estimated 7,500 tons in 1983 (ARB 1989). DCM is used as a solvent in the production of polycarbonate resins and triacetate fibers, in film processing, in ink formulations, and in electronics manufacturing. DCM is used as a foam blowing agent; as a metal cleaning and finishing solvent; as a propellant in aerosols of paints, automotive products, and insect sprays; and as a solvent in adhesives and coatings (U.S. EPA 1994b). DCM was once registered in the U.S. for use as a post-harvest fumigant for grains and strawberries, and a degreening agent for citrus fruits. DCM is no longer used for decaffeination of coffee and extraction of spices and hops because of concerns about residual solvent (ATSDR 1993). The FDA (1989) has banned the use of DCM in hair sprays and cosmetic products.

DCM, with production and consumption far exceeding one million pounds annually in the U.S., is used in more than 10 industries. DCM is used in California in the same products and processes as in the rest of the U.S. (ARB 1989). It is used as an image photo stripper for circuit board manufacturing, as a cold cleaning and vapor degreasing solvent for electroplating, as a solvent for integrated iron and steel manufacturing, as a laboratory solvent for dilution, extraction, liquid chromatography, as a solvent for metal degreasing and paint stripping, as a solvent for paper coating, as a solvent for insecticide, photographic film coatings and pharmaceuticals manufacturing, as a blowing agent for urethane foam production, and as a resin solvent and a varnish solvent in wood stains and varnishes (EDF 1999).

In Canada, approximately 13,200 tonnes of DCM are used annually (Environment Canada 1993). In Japan, DCM was found to be the most widely used chlorinated hydrocarbon solvents for degreasing in a survey of 196 unit workers in 95 plants in 1994 to 1996 in Hiroshima Prefecture, repeated twice a year for three years (Yasugi et al. 1998). In Denmark, analyses of 82 hair-care

cosmetic products from 27 European companies revealed that less than 500 ppm were present in six products suggesting that the use of DCM in cosmetic products could be eliminated (Rastogi 1994).

DCM at low concentrations can prevent completely and reverse partially the aggregation of hemoglobin (Hb) (Collison et al. 1977). Metabolism of DCM to CO increased the CO affinity to Hb (Nunes and Schoenborn 1973). Unlike other halomethanes, reductive DCM metabolites did not exhibit haemoprotein loss *in vitro* (Manno et al. 1995). Red blood cells from homozygous sickle cell patients exposed to DCM showed that DCM prevented sickle cell formation *in vitro* and reversed sickle cells to normal (Matthews et al. 1977, Schoenborn 1976). Therefore, as one of the small molecules with high affinity for specific sites in heme proteins, DCM was proposed to be a candidate for the treatment of sickle cell anemia. It was suggested that in the search for useful therapeutic agents attention be given to these non-heme ligands of Hb (Milosz and Settle 1975).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Occurrence

DCM has no known natural sources. Its entry into the environment occurs during production, transportation, use, and storage, as well as from waste disposal sites, industrial effluents, and water treatment facilities (U.S. EPA 1985a). DCM is mainly emitted into the air by a variety of stationary sources in California (ARB 1989). The U.S. EPA (1990) Toxics Release Inventory (TRI) listed 1,475 industrial facilities in the U.S. that produced, processed, or otherwise used DCM in 1988, and their estimated releases of DCM to the environment was about 127 million pounds or 57.7 million kg in total. In addition, an estimated 243 million pounds of DCM were released in 1988 from consumer products and hazardous waste sites. DCM was the 14th largest release and transfer chemical reported in the U.S. for 1988 (U.S. EPA 1990). In 1989, it was estimated that 30.7 million kg of DCM were released into the air and 0.1 million kg into surface water, 0.75 million kg were injected into underground wells, and 0.67 million kg were sent to U.S. landfills and other treatment or disposal facilities. The estimated industrial release was about 45.4 million kg in 1989 and 42.5 million kg in 1990. In 1992, about 74 million pounds were released to the atmosphere, about 221,000 pounds to surface water, about one million pounds to underground injection, and about 79,000 pounds to land from industrial sources. In 1997, TRI reported that among the total environmental releases of more than 47.5 million pounds of DCM, about 46.9 million pounds were released to the atmosphere, about 9,438 pounds to surface water, about 528,026 pounds to underground injection, and about 11,180 pounds to land from industrial sources (EDF 1999).

As much as 80 to 100 percent of the DCM used may be released into the environment because it is dispersed when it is applied (ARB 1989). DCM has been detected in indoor air, outdoor air, and surface water across Canada (Environment Canada 1993, Long et al. 1994). Of the total DCM released in the U.S., about 86 to 95 percent is released to the atmosphere, about 2 to 12 percent to land, about 2.4 percent to groundwater, and about 0.3 percent to surface water (ATSDR 1993). Levels of DCM measured were reported to be about 6.7 $\mu g/m^3$ (about 2 ppb) in urban U.S. air, about 39 $\mu g/m^3$ (about 11 ppb) in air around hazardous waste sites, about 1 to 50 $\mu g/L$ (ppb) in surface water with a maximum of 743 ppb in surface water samples of a New Jersey monitoring project, about 3,600 ppb in groundwater, about 13 $\mu g/kg$ in sediment, and about 1 to 310 $\mu g/kg$ in lipophilic food samples (ATSDR 1993). DCM levels in air, water, soil,

sediment, and tissues are most often determined by gas chromatography combined with mass spectrometry with electron capture detection or flame ionization detection. Reported detection limits are as low as 0.1 $\mu g/m^3$ (about 0.03 ppb) in air, 0.03 $\mu g/L$ (ppb) in water, and 5 $\mu g/kg$ dry weight in soil (Environment Canada 1993).

The primary pathway of DCM removal from surface water is by evaporation with a half-life of about three to six hours. Biodegradation has been demonstrated by microorganisms and by reactions with other chemicals in soil and in natural waters ranging from about six hours to seven days (Freedman et al. 1997). Neither oxidation, hydrolysis, bioaccumulation in aquatic plants or animals or organisms, or adsorption to soils and sediments play significant roles in removing DCM from water. Half-life of DCM in water ranges from one to six days. DCM in the atmosphere is degraded by reactions with photochemically produced hydroxyl radicals with a half-life ranging from 53 to 127 days (ATSDR 1993). It is not subject to direct photolysis. DCM spilled on or into soil evaporates rapidly from surface soil and partially leaches into nearby groundwater where its fate is unknown (Howard 1990). More than 90 percent of the DCM in the environment degrades to CO_2 (ATSDR 1993).

Air, Soil, Food, and Other Sources

DCM is found mostly in air because of its volatility. Background level in air was reported as about 0.17 $\mu g/m^3$ or about 0.05 ppb. Ambient air in a highly urbanized area may contain an average DCM concentration of about 3.7 ppb. DCM was detected in U.S. rural area air at mean concentrations of about 0.18 to 2.1 $\mu g/m^3$ or about 0.05 to 0.6 ppb. It was found in urban air in the U.S. at mean concentrations of about 0.8 to 6.7 $\mu g/m^3$ or about 0.2 to 2 ppb with maximum concentrations of 22 to 200 $\mu g/m^3$ or about 6 to 60 ppb. DCM was in the air of at least 746 of 1,300 National Priorities List hazardous waste sites identified by the U.S. EPA. Mean air concentrations detected at some hazardous waste sites were about 0.3 to 39 $\mu g/m^3$ or about 0.1 to 11 ppb. Some nonresidential indoor air contained DCM with mean concentrations of 0.2 to 19,000 $\mu g/m^3$ or about 0.06 to 5,600 ppb (ATSDR 1993). DCM contributes to indoor air pollution due to its use in consumer products, building materials and furnishings (EDF 1999). DCM was detected in gas from wells in four municipal solid waste landfills in southern Finland (Assmuth and Kalevi 1992).

More than 90 percent of the DCM in air comes from industry and consumer products. DCM is formed during water chlorination and air emissions of DCM from wastewater treatment plants in California exceeded 400,000 pounds in 1987 (NRC 1977, ATSDR 1993). In occupational environment, DCM concentrations in air ranging from 3,500 to 35 million $\mu g/m^3$ have been detected in general work areas, while concentrations as high as 49 million $\mu g/m^3$ have been reported in samples in the breathing zone of some workers. Indoor air concentrations resulting from the use of DCM containing consumer products were estimated to range from 0.21 to 19,000 $\mu g/m^3$. Before the ban of DCM in consumer products by FDA in 1989, a concentration of 50,000 ppb or 174,000 $\mu g/m^3$ in the breathing zone of consumers would be expected following hair spray use resulting in a TWA exposure of 174 ppb; hair care specialists might be exposed to ten-times this level (ATSDR 1993).

Based on its gas phase reactivity with hydroxyl radicals, DCM has an atmospheric lifetime estimated to range from 80 to 250 days (ARB 1989). The principal releases of DCM to land are from the disposal of DCM products and containers in landfills. When DCM is spilled on land, it attaches loosely to nearby surface soil particles and mostly moves from soil to air with some into groundwater. Due to its high volatility, water and food concentrations of DCM are relatively

small. DCM was detected in 20 percent of 338 sediment samples recorded in the U.S. EPA STORET database with a median concentration of 13 μg/kg (ATSDR 1993).

A Total Diet study by FDA in 1987 showed that eight out of 19 table-ready foods contained DCM above the quantitative detection limit; for example, all seven butter samples tested contained 1.1 to 280 μg DCM per kg sample, all seven margarine samples contained 1.2 to 81 μg/kg, ten of eleven cereal samples contained 1.6 to 300 μg/kg, all eight cheese samples contained 3.9 to 98 μg/kg, four of seven peanut butter samples contained 26 to 49 μg/kg, and ten of twelve frozen food samples contained 5 to 310 μg/kg. No DCM was detected in ice cream and yogurt samples (OECD 1994, IPCS 1996). Residual levels of DCM in decaffeinated coffee beans ranging from 0.32 to 0.42 mg/kg were reported in the U.S., well within the FDA limit of 10 ppm or 10 mg/kg (IARC 1986). The concern about residual DCM in decaffeinated coffee does not exist since coffee companies no longer use DCM. Mean DCM levels in oysters and clams from Lake Pontchartrain in Louisiana were reported as 7.8 and 27 μg/kg, respectively (ATSDR 1993).

Water Sources

Although DCM has limited water solubility, it is found in surface water, groundwater, finished drinking water, commercially bottled artesian water, and surface water sites in heavily industrialized river basins. Chlorination in treatment plants is also a source of DCM in drinking water supplies. The principal releases of DCM to surface water and potentially to groundwater are via industrial effluents and underground injections, respectively. Due to its low solubility, water has been considered as a minor source of DCM occurrence in the environment (NRC 1977, ATSDR 1993). DCM was the sixth most frequently detected organic contaminant in groundwater from hazardous disposal sites investigated in 1987; it had a detection frequency of 19 percent (ATSDR 1993). It is estimated that about 10 percent of the total DCM used in the U.S. is released to water (Cothern et al. 1984).

The mean concentrations of DCM in drinking water are generally detected at less than one ppb. Samples from 128 U.S. drinking water wells showed that 3.1 percent of them had levels of 1 to 5 ppb DCM. DCM was detected in 30 percent of 8,917 surface water samples recorded in the U.S. EPA STORET database with a median concentration of 0.1 ppb. It was found in 45 percent of 605 surface water samples in a New Jersey survey with a maximum concentration of 743 ppb (ATSDR 1993). In a drinking water survey from 10 U.S. states, two percent of samples were detected with DCM with a maximum of 3,600 ppb from groundwater sources and 13 ppb from surface water sources. In an U.S. EPA Region V survey of 83 sites in Indiana, Illinois, Minnesota, Ohio, Wisconsin, eight percent of samples were positive with 1 to 7 ppb DCM. In the 1976 National Organics Monitoring Survey, 15 of 109 samples were positive with a mean of positive samples of 6.1 ppb (Howard 1990).

In 1983, DCM was discharged into Detroit River, Ontario, Canada at daily rates of 0.03 to 0.31 kg in effluents from automotive painting operations, steel manufacturing, and sewage treatment plants (Environment Canada 1993). In a Canadian study of 30 potable water treatment facilities, averages of 3 ppb in the winter and 10 ppb in the summer and a maximum of 50 ppb were observed with 50 percent positive results (Howard 1990, OECD 1994, IPCS 1996). In a survey of 182 samples of retail bottled water purchased in Canada, DCM was detected in four samples with an average concentration of 59 μg/kg ranging from 22 to 97 μg/kg (Page et al. 1993). DCM was detected in groundwater in Germany with other organochlorine solvents with approximately 25 percent of the samples containing more than one ppb of a single solvent (Dieter and Kerndorff 1993).

Of the 1,588 surface water sites sampled from 33 states not including California from 1988 to 1991, 81 sites or 5.1 percent had detectable DCM. In the same study, 294 sites or 2.4 percent of the 12,263 groundwater sites had detectable DCM. Of the 41,506 drinking water sites sampled from 11 states including California from 1992 to 1994, 1.26 percent had detectable DCM but 1.13 percent were below the MCL of 5 ppb and only 0.13 percent were above the MCL (U.S. EPA 1997). Averages of 68 ppb DCM in surface water and 98 ppb in groundwater have been found at some hazardous waste sites (ATSDR 1993).

In 1985, DHS conducted a statewide monitoring program, as mandated by California Assembly Bill 1803 (AB1803), for toxic organic chemicals in groundwater in large water systems that are defined as systems with greater than 200 service connections (DHS 1986). Among 2,949 wells analyzed for toxic chemicals, DCM was detected in 11 wells, that is, 10 in Los Angeles County and one in Riverside County, at mean concentrations ranging from 0.7 to 10 ppb. A private well with contamination from nearby McClellan Air Force Base had levels of 96 ppb of DCM.

From 1985 to 1990, 4,765 wells in small water systems which are systems with less than 200 service connections were sampled under the AB1803 program. DCM was found in one well in Inyo County and one well in Los Angeles County at a mean concentration of 2.2 ppb and 1.3 ppb, respectively (DHS 1990). From 1984 to 1998, DCM was detected in 68 sources among 14,092 sampled sources with a detection limit for purposes of reporting (DLR) of 0.5 ppb as reported by DHS at http://www.dhs.cahwnet.gov/ps/ddwem/chemicals/phgs/mclcomparison.htm on April 25, 2000. DCM was one of the abundant contaminants frequently detected in groundwater at 43 Finnish landfills (Assmuth and Strandberg 1993).

Exposure

DCM has been recognized as one of the 13 most important chlorinated aliphatic hydrocarbons from the standpoint of industrial hygiene since as early as 1936 (Lehmann and Schmidt-Kehl 1936). Exposures to DCM happen mostly from breathing contaminated air. The highest and most frequent exposures usually occur in workplace where DCM is used. The principal route of exposure for the general population to DCM is inhalation of ambient air. Non-occupational inhalation exposure also occurs through the use of consumer products containing DCM (ECETOC 1994). Ingestion of water and food is also considered as a major route of potential human exposure. Dermal absorption has been observed, although it occurs more slowly than absorption after ingestion or inhalation (ATSDR 1993).

Breath analysis can be used to monitor DCM exposure (Stewart et al. 1976). U.S. EPA has estimated that over one million workers are currently exposed to DCM (NCI 1975). The National Occupational Hazard Survey, conducted by NIOSH (1976) from 1972 to 1974, estimated that 2.5 million workers were potentially exposed to DCM vapors. OSHA (1997) estimated that over 237,000 workers exposed to DCM in over 91,000 DCM-using establishments are at risk of developing cancer, heart and liver effects, and nervous system impairments, as well as eye, skin, and mucous membrane irritation. Approximately 20.3 million people in California are estimated to be exposed to a population-weighted mean DCM concentration of 1.1 to 2.4 ppb in the ambient air (ARB 1989).

From the archives of the Danish National Institute of Occupational Health, DCM was measured in 208 air samples with a mean concentration of 51 mg/m^3 during the years of 1955 to 1988 (Jensen et al. 1990). A survey of occupational carcinogens by the Institute of Occupational Health, Finland (Anttila et al. 1993) showed that among the 17,118 registered workers about

2,000 workers in paint removal or pharmaceutical industries were exposed to DCM at an estimate

of TWA of 10,000 μg/m^3 in 1989. In France, the exposure of workers to DCM in an aeronautical workshop during stripping of paint from a Boeing B747 calculated for an eight-hour workday ranged from 86 to 1239.5 mg/m^3, equivalent to about 23.9 to 344.3 ppm. DCM air concentrations ranged from 299.2 to 1888.9 mg/m^3, equivalent to about 83.1 to 524.7 ppm (Vincent et al. 1994). DCM was identified as a major solvent in 22 solvent products used in Italian shoe factories (Perbellini et al. 1992).

Monitoring data for DCM in workplace air from 1968 to 1982 indicated that concentrations in the general work area ranged from 300 to 3,350,000 μg/m^3, while samples in the breathing zone of workers ranged up to 4,900,000 μg/m^3 (IARC 1986). DCM exposures among furniture stripping workers were reported ranging from 600 to 1,500 ppm, equivalent to about 2,100,000 μg/m^3 to 4,025,000 μg/m^3; the levels were lowered to about 30 ppm (approximately 105,000 μg/m^3) with the installation of a ventilation system (Estill and Spencer 1996). DCM was detected in almost all spacecraft air samples in at least trace quantities with a highest concentration of 500 μg/m^3 (James 1997). Monitoring DCM exposed workers including 12 smokers and eight nonsmokers showed a correlation in nonsmokers between DCM concentration in air and CO concentration in alveolar air, and a correlation between DCM in breathing zone and in urine (Ghittori et al. 1993).

The average daily intake of DCM from outdoor air in three U.S. cities was estimated to range from 33 to 309 μg/day based on 1979 monitoring data (ATSDR 1993), and from outdoor air in general was 28 to 268 μg/day assuming air concentrations of 0.4 to 3.8 ppb (Howard 1990). The average daily intake of DCM from water, assuming 0 ppb for 86 percent population and 6.1 ppb average for 14 percent population, was estimated to range from 0 to 12.2 μg/day for an adult drinking two liters of water per day (Howard 1990).

The daily intake of DCM by the Canadian population was estimated to range from 0.04 to 0.46 μg/kg/day from ambient air, 3.88 to 6.04 μg/kg/day from indoor air, 0 to 0.07 μg/kg/day from drinking water, and 0.03 to 0.11 μg/kg/day from food. The total intake of various age groups was estimated to range from 3.96 to 4.28 μg/kg/day for newborn to six-month-old, 5.39 to 5.77 μg/kg/day for seven-month to four-year-old, 6.2 to 6.62 μg/kg/day for 5- to 11-year-old, 5.11 to 5.45 μg/kg/day for 12- to 19-year-old, and 4.56 to 4.86 μg/kg/day for 20-year and older (Canadian Environmental Health Directorate 1981, Environmental Canada 1993, Long et al. 1994).

A preliminary exposure assessment of DCM in Japan was made. Concentrations for humans were about 3×10^{-4} μg/L in inhaled air, 0.116 μg/L in drinking water, 0.058 μg/kg in fish, 1.9×10^{-5} μg/kg in meat, 1.6×10^{-5} μg/kg in milk, and 1.33 μg/kg in vegetables. Daily dose of DCM exposure was about 3.28 μg/day by inhalation, 0.12 μg/day from drinking water, 0.01 μg/day from fish, zero from meat and milk, and 0.5 μg/day from vegetables (Yoshida 1993).

Placental transfer of DCM was reported in pregnant Sprague-Dawley rats and humans. Following inhalation of 500 ppm DCM for one hour, the mean concentration of DCM in maternal and fetal blood in rats was 176 ± 50 and 115 ± 40 nmole/mL, respectively (Anders and Sunram 1982). DCM, measured in 17-day-old rat fetuses after maternal inhalation exposure to concentrations between 107 and 2,961 ppm for five hours, showed a dose-related increase ranging from 0.87 to 36.8 μg/g (Withey and Karpinski 1985). DCM was found in the placenta, fetus and breast milk of Russian women occupationally exposed to an air concentration of 86 mg/m^3 or approximately 24 ppm. A mean concentration of 0.07 mg DCM/L breast milk was reported (Vozovaya et al. 1974, OECD 1984, Tabacova 1986, Sullivan et al. 1993).

Humans can be exposed to DCM via inhalation, ingestion, and dermal contact. As described later in this report, to assess human exposures and doses attributable to DCM in water, these three exposure routes were used to derive "pathway-specific absorbed dose-rates" (PAD) for the "best"

and "upper bound" estimates of cumulative exposures based on quantitative relations in the physiological constitutions of mammals for the purpose of deriving public health protective concentrations in drinking water (Adolph 1949). Inhalation is the principal route of human exposure to DCM. The lungs and the gastrointestinal tract readily absorb DCM. Experimental studies indicate that DCM is absorbed slowly across intact human skin. Permeation of protective clothing materials by DCM was reported with breakthrough times in the range of two to eight minutes (Vahdat 1987).

METABOLISM AND PHARMACOKINETICS

IARC (1999) in its most recent review concluded "Mechanistic studies have established a link between GST-mediated metabolism of DCM and its genotoxicity and carcinogenicity in mice." The European Chemical Industry Ecology and Toxicology Center (ECETOC 1984, 1987, 1988, 1989) published an overview of experimental work investigating DCM metabolism and carcinogenicity as well as U.S. EPA (1985a) and OSHA (1997). Parameters of comparative pharmacokinetics of DCM were determined after intravenous injection to male Wistar rats at five doses (Withey and Collins 1980). Metabolic rate constants for DCM were determined in Sprague-Dawley rats (Kim et al. 1996a).

DCM is absorbed through the lungs, gastrointestinal tract, and skin. Once it enters the body, more than 70 percent of DCM enters the bloodstream and is distributed throughout the body. Most of the DCM goes to the liver, kidney, brain, lung, and fatty tissue with preferential deposition in adipose tissue. Increased physical activity, or an increased amount of body weight or body fat tends to increase the amount of DCM that remains or accumulates in the body tissue (Dhillon and Von Burg 1995, OEHHA 1992). DiVincenzo et al. (1972) found that about 40 percent of the absorbed DCM is not eliminated in the expired air in humans and canines exposed to DCM vapor. This remaining amount of DCM is partly metabolized to CO and has a CO excretion half-life of approximately 13 hours, versus the excretion half-life of 5.2 hours of inhaled CO. DCM is unreactive in the body until it is metabolized. About half of the DCM in the blood leaves within 40 minutes (ATSDR 1993).

The uptake, metabolism and excretion of DCM was modeled using data from eleven men exposed to 50, 100, 250, and 500 ppm, and nine women to 250 ppm, for 1, 3, or 7.5 hours per day, up to five consecutive days (Peterson 1978). In Japan, a preliminary exposure assessment of DCM in humans estimated 2.28 ng/minute as the inhaled mass, 0.43 ng/minute as the ingested mass, 1.02 ng/minute as the exhaled mass, and 1.69 ng/minute as the metabolized mass. Steady state mass balance concentration in humans was about 0.3 ng/L in inhaled air, 0.14 ng/L in exhaled air, 1.31 ng/L in arterial blood, 1.11 ng/L in venous blood, 0.72 ng/L in liver, 16.3 ng/L in fat tissue, 1.92 ng/L in rapidly perfused tissues, and 1.08 ng/L in slowly perfused tissues (Yoshida 1993).

Available data suggest that there are two pathways by which DCM is metabolized, the mixed function oxidase (MFO) enzyme pathway associated with the microsomal cell fraction, and the pathway involving glutathione sulfotransferase (glutathione-S-transferase, GST or GSHT) located primarily in the cytoplasm. In humans and some experimental animals, the MFO pathway seems to be the preferred detoxification pathway for DCM following inhalation or oral exposure. Many investigators believe that one or more of the metabolites of DCM, and not DCM itself, is responsible for its genotoxicity and carcinogenicity (OSHA 1997). The glutathione-dependent metabolism has been held responsible for the tumors observed in mice (ECETOC 1989). The GST pathway has been postulated to be the one leading to DCM carcinogenesis (Andersen et al. 1987a).

Biological oxidation of ^{14}C-DCM to CO and CO_2 by the rat (Rodkey and Collison 1977), by the perfused rat liver (Takano and Miyazaki 1988), and by isolated rat hepatocytes (Stevens et al. 1980) was reported. Unchanged DCM and its metabolites are excreted primarily in expired air as CO and CO_2, with urinary excretion accounting for less than nine percent of the dose after inhalation or oral exposure. The excretion usually occurs within 48 hours after DCM exposure (Ahmed and Anders 1976, 1978, Kubic and Anders 1975, 1978, Kubic et al. 1974). Elevated COHb levels were found in workers exposed to DCM vapor with an average peak blood COHb saturation of 3.9 percent (Sullivan et al. 1993).

Absorption

DCM may enter the body by a number of routes through the skin, the lung, or the intestine. Inhalation is the primary and ingestion is the secondary route of exposure in humans. In establishing the scientific basis for Swedish occupational standards, it was reported in 1981 that most uptake with occupational exposure to DCM is via the respiratory organs. This is about 50 to 55 percent of the inhaled amount while at rest, about 40 percent with light work, and about 25 percent with heavy physical labor (RSC 1986).

DCM is readily absorbed in rats after inhalation or oral administration, and to a lesser extent through the skin. Under non-equilibrium exposure conditions, absorption of DCM has been estimated at 31 to 75 percent of an inhaled dose in both humans and experimental animals. Following oral intake of aqueous solution, DCM is rapidly absorbed at the rate of 92 to 96 percent or even up to 100 percent within 20 minutes in the gastrointestinal tract of animals. The absorption is only about 55 percent after oral intake of DCM in a hydrophobic vehicle. Reports of human poisoning cases indicated considerable absorption across the gastrointestinal tract of humans. Dermal absorption of DCM has been estimated roughly at about one to six percent based on limited information.

Pulmonary Absorption

Inhalation is the principal route of human exposure to DCM. It is the most significant route of entry for DCM in occupational settings. In humans, it was estimated that about 70 to 75 percent of inhaled DCM was readily absorbed across the surface of the lungs (DiVincenzo and Kaplan 1981a). The human blood to air partition coefficient of DCM has been estimated to be between 6.5 and 9.7 (Andersen et al. 1987a, ECETOC 1988, Fiserova-Bergerova 1976, Gargas et al. 1989, Lindquist 1978, Morgan et al. 1972). Pulmonary absorption of DCM is characterized by an initial period of rapid uptake; during the first hour of exposure to 100 to 200 ppm, the blood concentration reaches approximately 0.6 mg/L. At 50 ppm exposure, the increase in blood DCM concentration was about 0.2 mg/L during the first hour (DiVincenzo and Kaplan 1981a).

The amount of DCM that initially enters arterial blood depends primarily on the concentration difference between alveolar and venous blood. With continued exposure, the net uptake of DCM decreases as tissue concentrations begin to equilibrate with DCM in alveolar air (Astrand et al. 1975, DiVincenzo et al. 1972, DiVincenzo and Kaplan 1981a, Riley et al. 1966). DCM concentrations in blood reached steady state after about two to four hours of inhalation exposure. The constant correlation between the steady state blood DCM values and the exposure concentrations was approximately 0.008 ppm in blood per one ppm in air (DiVincenzo and Kaplan 1981a, 1981b, McKenna et al. 1982).

Like other lipophilic organic vapors, DCM absorption appears to be influenced by factors other than the vapor concentration. Pulmonary uptake of DCM depends on the concentration in inspired air, the breathing rate, the duration of exposure, the solubility of DCM in blood and tissue, the volume and rate of perfusion of tissues, the rate of elimination, and the rate of alveolar ventilation (U.S. EPA 1985a, OSHA 1997). Increased physical activity increases the amount of DCM absorbed by the body due to an increase in ventilation rate and cardiac output, since these factors increase blood flow through the lungs and promote absorption (Astrand et al. 1975, DiVincenzo et al. 1972). Uptake also increases with the percent body fat. Under controlled conditions, there was a 30 percent greater absorption and retention of DCM by obese subjects exposed to 75 ppm or about 260 mg/m^3 for one hour as compared to lean subjects (Engstrom and Bjurstrom 1977). Respiratory uptake of DCM was influenced by P450 inhibitors in male B6C3F1 mice (Ottenwalder et al. 1989).

Blood DCM measurements during inhalation exposures of animals to DCM indicate that absorption is proportional to the magnitude and duration of the exposure over a DCM concentration range of about 100 to 8,000 ppm or about 350 to 28,000 mg/m^3 (DiVincenzo et al. 1972, Green et al. 1988, MacEwen et al. 1972, McKenna et al. 1982). As was the case with humans, blood DCM levels reached steady state as the duration of exposure increased in dogs and rats (DiVincenzo et al. 1972, McKenna et al. 1982). Data from studies of rats during six-hour exposures to 50, 500, and 1,500 ppm suggest that the steady state blood to air concentration ratio increases with exposure concentration from 0.001 to 0.005 and 0.007, respectively (McKenna et al. 1982). It is postulated that the increased ratio at steady state results from saturation of metabolic pathways as exposure increases rather than from an increased absorption coefficient.

Intestinal Absorption

No experimental studies have been conducted on humans to evaluate the extent of absorption of DCM after ingestion, but it was estimated from studies of poisoning incidents that considerable gastrointestinal absorption of DCM occurs in human (U.S. EPA 1985a). A 38-year-old male became deeply unconscious within 1.5 hours after ingestion of one to two pints of a paint remover, equivalent to about 9,000 to 18,000 mg DCM per kg (Roberts and Marshall 1976). A 56-year-old female smoker was unconscious with DCM and methanol in her blood within an hour after ingestion of approximately 300 mL of a paint stripper (Hughes and Tracey 1993).

DCM in water is absorbed rapidly across the gastrointestinal tract of animals, and is detected in the systemic circulation within minutes after oral administration (U.S. EPA 1985a). Rats given 1 or 50 mg DCM in water per kg by gavage, excreted 92 and 96 percent, respectively, of the administered radiolabeled DCM as CO and CO_2, approximately 15 minutes after dosing (McKenna and Zempel 1981). Similar results were obtained by Angelo et al. (1986a, 1986b) who exposed rats and mice to ^{14}C-DCM in water at 50 or 200 mg/kg by gavage. Ten minutes after treatment of 50 mg DCM in water per kg by gavage, about 24 percent of the administered dose were recovered from the upper gastrointestinal tract of the mice. In total about 75 percent of the dose was absorbed within 10 minutes and about 98 to 100 percent of the dose within 20 minutes (Angelo et al. 1986a, 1986b).

On the other hand, the gastrointestinal absorption of DCM is slowed significantly by the use of corn oil as a vehicle which is hydrophobic, compared to water. During a two-hour observation, approximately 40 to 45 percent of the 50 mg dose was absorbed from the oil as compared to essentially all of a comparable dose in water (Withey et al. 1983). Only approximately 55 percent of the administered dose of 10 to 1,000 mg in corn oil per kg was detected in the stomach

and small intestine after 20 minutes and remained there at two hours. The large intestines and caecum

tissues and contents contained about five to eight percent of the dose at 10 minutes. This value declined to one to two percent at the end of two hours (Angelo et al. 1986a).

Dermal Absorption

The extent of uptake through the skin, under conditions of environmental exposure to low aqueous concentration of DCM, is unknown. Experimental studies indicate that liquid DCM is absorbed slowly across the human skin (Kelley 1988, Stewart and Dodd 1964). Permeability rate of DCM through living human skin was measured as 24 g/m^2-hour (Ursin et al. 1995). However, concentrations were not provided and the rate of dermal absorption was estimated by the mean peak concentration of DCM in alveolar air.

Substantial occupational exposure could occur through the dermal route when the worker is exposed to high concentrations of DCM vapor without wearing adequate protective clothing. Trapping DCM against the skin with clothing or gloves over periods of time can lead to greater dermal absorption. One example by PBPK modeling indicated that sufficient DCM might be absorbed by the dermal route over an eight-hour shift to give an internal concentration which would exceed that experienced by workers exposed to DCM through inhalation of 25 ppm for eight hours (OSHA 1997).

Among animal species, dermal absorption of DCM has only been measured in rats. The mean dermal permeability constant obtained for rats exposed to 30,000, 60,000, and 100,000 ppm in air was 0.28 cm/hour. The total amount absorbed was estimated to be 34.4, 57.5, and 99.4 mg, respectively, for the three concentrations tested (McDougal et al. 1985, 1986). The skin to air partition coefficient for DCM was 13.6 ± 0.5 based on 17 male Fischer 344 rat samples measured at 203 ppm and four samples measured at 41 ppm with an equilibration time of two hours. The skin to air partition coefficient values for the 12 volatile chemicals measured correlated with previously determined vapor permeability constants but correlated poorly with octanol-water partition coefficient values (Mattie et al. 1994).

Distribution

DCM, absorbed through the lungs, gastrointestinal tract, and skin, is distributed throughout the body with preferential storage in adipose tissue (Davis and Mapleson 1981, Thier et al. 1991). The human fat to blood partition coefficient has been estimated to be 8.8 (ECETOC 1988). The extent to which DCM is stored in body tissue is not yet known, but DCM-derived CO has an effective half life of up to 13 hours in workers exposed to about 180 ppm DCM, about 2.5 times that of exogenously inhaled CO at 35 or 50 ppm (Nager and O'Connor 1998, Ratney et al. 1974).

Autopsies performed on victims of fatal overexposure to DCM have found DCM in the liver, kidney, and brain (Bonventre et al. 1977, Moskowitz and Shapiro 1952). DCM was detected in the breast milk, at a mean level of 74 ppb in 17 of 28 samples approximately five hours after start of work and lower levels after termination of work, of Soviet women exposed to DCM at a rubber product manufacturing plant (Vozovaya et al. 1974). DCM was detected in all eight samples of human milk from four urban areas. Whole blood samples from 250 subjects had DCM levels from non-detectable to 25 ppb with an average of 0.7 ppb (Howard 1990, NTP 1986).

Because of the tendency of DCM to partition to fat, the total uptake and retention of DCM is dependent on the relative size and obesity of human subjects (Engstrom and Bjurstrom 1977). In

exposure to 2,600,000 $\mu g/m^3$ or about 750 ppm DCM for one hour while exercising on a bicycle ergometer, obese individuals among 12 healthy males aged 21 to 35 retained approximately

30 percent more DCM than slender individuals. The mean concentration was 10.2 mg/kg one hour after exposure and 8.4 mg/kg after four hours with a wide distribution. Two obese subjects had 1.6 and 1.7 mg/kg 22 hours after exposure.

In studies using radiolabel DCM given as a single gavage dose, 50 or 100 mg/kg, radioactivity was detected in the liver, blood, lungs, heart, spleen, bone marrow, salivary glands, and pancreas of rats and mice (Kirschman et al. 1986). Similar widespread distribution of DCM was found in rats following inhalation or intraperitoneal exposures (Carlson and Hultengren 1975, DiVincenzo and Hamilton 1975, McKenna et al. 1982). In rats exposed to 200 ppm DCM for six hours per day for four days, the amount of DCM present in perirenal fat was approximately five to six times greater than that detected in other tissues (Savolainen et al. 1977). DCM crosses the placenta, and has been measured in the fetuses of rats exposed to DCM by inhalation (Anders and Sunram 1982, Schwetz et al. 1975).

Metabolism

Metabolism of DCM is critical for its toxicity and detoxification in the body (Bus and Reitz 1992). Toxicokinetics is an analytical tool to quantify the reactive intermediates in target tissues for assessing DCM hazards to man (Clewell et al. 1988, Reitz 1991b). In general, metabolism *in vivo* may vary according to the age and species of the animal, or as a result of the effects of the chemical on its own metabolism, or as a result of the toxic properties of the chemical (Green 1989, 1990a, 1990b, 1991, Green et al. 1986a, 1986b, Schumann et al. 1984). Experiments with ^{14}C-DCM indicated that absorption from the gastrointestinal tract of rats and mice was rapid and that the liver was the major site of metabolism (Gargas et al. 1986, Reitz et al. 1986, 1989). Two pathways metabolize DCM. The saturable MFO pathway generates CO and CO_2 while the GST pathway generates CO_2 (Reitz 1991a). An elevated carboxyhemoglobin (COHb) level has been reported following human exposure to DCM via inhalation (Horowitz 1986) or ingestion (Hughes and Tracy 1993), and following inhalation (Kurppa and Vainio 1981), oral (Stewart and Hake 1976), or intraperitoneal (Hogan et al. 1976, Yesair et al. 1977) administration of DCM in rats.

DCM metabolism is influenced by pretreatment or simultaneous administration of other chemicals. As measured by the DCM-induced COHb concentrations in rats, the DCM metabolism is affected by acute oral or inhalation exposure to substrates of cytochrome P450 enzymes such as acetylsalicylic acid (Pankow et al. 1994); aromatic hydrocarbons (Pankow et al. 1991a); benzene, toluene, or m-xylene (Kim and Kim 1996); ethanol (Glatzel et al. 1987, Pankow et al. 1990); isoniazid (Pankow 1988, Pankow et al. 1992); methanol (Pankow and Jagielki 1993); phenobarbital (Pankow et al. 1992); styrene (Mortensen et al. 1998); pyrazole (Pankow et al. 1991b); or toluene (Krishnan and Pelekis 1995, Toftgard et al. 1982). This indicates that cytochrome P450 2E1 enzyme is involved in the metabolic conversion of DCM.

In rats receiving high oral doses of DCM and toluene that are metabolized by the same isoenzyme P450 2E1, reduced COHb was observed due to noncompetitive and uncompetitive metabolic inhibition (Krishnan and Pelekis 1995, Pelekis and Krishnan 1997). Wirkner et al. (1997) demonstrated the stimulating effect of long-term ingestion of 10 percent ethanol for 4, 12, and 36 weeks on the metabolism of inhaled or ingested DCM to CO as measured by COHb in male Wistar rats. Concomitant intraperitoneal treatment with a nonhepatotoxic dose of DCM at 6 mmole/kg, which increased the covalent binding of [^{14}C]C^{14} metabolites to microsomal lipids, potentiated the hepatotoxicity of carbon tetrachloride at 2 mmole/kg in rats (Kim 1997). Inhalation exposure in guinea pigs to approximately 500 ppm of DCM for five days with high

concentrations of ethanol potentiated the liver toxicity of DCM, however, exposure for one day suggested an antagonism between the two agents (Balmer et al. 1976).

Metabolic Pathways

DCM is metabolized both *in vivo* and *in vitro* by two separate metabolic pathways: one utilizes the cytosolic GST that requires the substrate glutathione to produce CO_2 and other metabolites, and the other utilizes microsomal cytochrome-P450-dependent mixed-function oxidase (MFO) to produce CO, CO_2 and other metabolites. Using a PBPK model, DCM pharmacokinetics was simulated (Angelo and Pritchard 1984). Metabolism of DCM takes place in the liver and lungs, but the liver appears to be the principal organ of metabolism (U.S. EPA 1985a). It has been postulated that the GST pathway contributes mainly to the carcinogenicity of DCM in B6C3F1 mice (Casanova et al. 1992, 1996, 1997; Casanova and Heck 1996; Dekant and Vamvakas 1993; OSHA 1997). The MFO pathway seems to be the preferred detoxification pathway for DCM metabolism following inhalation or oral exposures in humans and animals. No data were available on the metabolism of DCM after dermal administration.

CO and CO_2 are the only known tertiary metabolites of DCM. CO is produced solely by MFO whereas CO_2 is believed to be an end product of both the MFO and GST pathways. The glutathione-mediated pathway is a low-affinity, high-capacity process while the MFO pathway is a high-affinity, low-capacity process (Ahmed and Anders 1976, 1978, Gargas et al. 1986). Both pathways are thought to produce one or more reactive metabolites capable of binding to macromolecules; however, no intermediates have been isolated or identified *in vivo* (Ahmed et al. 1980, U.S. EPA 1985a, 1985b, 1987b). The reaction product of DCM with glutathione appears to be the causative agent for toxicity in rats and mice based on research on methyl chloride (also known as monochloromethane or chloromethane), a gas related to DCM (Chellman et al. 1986, Dodd et al. 1982)

There appear to be species differences in pathway preference. The GST pathway is more active in the mouse than in the rat, hamster, and human (ATSDR 1993). In studies of metabolic end products in rats and mice exposed to 500, 1,000, 2,000, and 4,000 ppm DCM for six hours, the cytochrome MFO pathway was saturated at ambient concentrations less than the 500 ppm exposure level (ATSDR 1993, Green 1989, McKenna et al. 1982). In the rat, after saturation of this pathway, little further metabolism of DCM occurred with increased dose. In contrast, significant metabolism of DCM occurred in the mouse at high dose by the GST pathway (Kirschman et al. 1986, McKenna et al. 1982). The MFO pathway was found to be saturable in rats and mice at inhalation exposures of greater than about 200 ppm over a six-hour period (Gargas et al. 1986). Saturation of the MFO pathway in humans has been estimated to occur at a level that is within the range of the animal data of 200 to 1,000 ppm. The GST pathway is not thought to be saturated for any of the species investigated at concentrations up to 4,000 ppm (OSHA 1997) or 10,000 ppm (Gargas et al. 1986).

Glutathione-S-Transferases (GST)

The GST pathway metabolizes DCM via a postulated S-chloromethylglutathionate conjugate in the cytosol (Dekant and Vamvakas 1993). Cytosolic metabolism of DCM, which is dependent on glutathione and mediated by the glutathione or GST, proceeds through the formation of a series of intermediates, and ultimately leads to the production of CO_2. [^{13}C]Formaldehyde hydrate, [^{13}C]S-(hydroxymethyl)glutathione, and [^{13}C]methanol were identified as metabolites of [^{13}C]DCM (Hashmi et al. 1994). Formaldehyde and chloride ions have been identified as products of GST-mediated metabolism of DCM *in vitro*, but not *in vivo*. However, metabolism is thought to progress via the formation of S-chloromethylglutathionate, formaldehyde, and formic

acid. Then formaldehyde spontaneously decomposes to CO_2, with some carbon entering the endogenous C-1 pool in mammalian systems (Ahmed and Anders 1976, 1978, Anders et al. 1977, Kubic and Anders 1975). Potential reactive metabolites in this pathway are the S-chloromethylglutathionate conjugate and formaldehyde which is known to react with protein, RNA and DNA (OSHA 1997). The glutathione-dependent pathway played a minor role in the metabolism of DCM in mice, at below 500 ppm through inhalation, similar to other species including humans (Ottenwalder et al. 1989).

The GST activities in subcellular fractions of lung and liver appeared to be different in three rodent species (Lorenz et al. 1984). The polymorphism of GST in gene structure, expression and chromosomal localization influences its activity towards DCM (Schroder et al. 1996, Whittington et al. 1999). In the lung and liver, B6C3F1 mice display a high rate of metabolism via the GST pathway. In contrast, there is a low rate in the rat, hamster and human tissues (Green 1997). Two GSTs that metabolize DCM, GST theta 1 (GSTT1) and GST theta 2 (GSTT2), were isolated from mouse, rat and human. Both enzymes were present at significantly higher concentrations in mouse tissues than in rat or human tissues (Mainwaring et al. 1996b). The two from mouse MT-1 (GSTT1-1) and MT-2 were found to be similar to the GST5-5 and GST12-12 purified from rat liver (Meyer et al. 1991). The greater conjugating activity seen in mice might be due to the difference in enzyme expression or to the significant contribution from MT-2 (Mainwaring et al. 1996a). *In vitro* recombinant human GSTT1-1 was found to conjugate glutathione with DCM but GSTT1-1 was found in low levels in soluble extract of the human pulmonary cells (Sherratt et al. 1997). The induction of GSTT1-1 by dietary components and its localization within cells increased bioactivation of DCM in rat liver (Sherratt et al. 1998). The rat liver GST5-5 activity towards DCM was reported as 11 μmole/minute/mg of protein (Meyer et al. 1991).

A GST (GSTT1) in human erythrocytes displays polymorphism and it appears that the human population can be divided into 1) high conjugators, a very small minority with especially high GST activity to catalyze the glutathione conjugation of DCM, 2) low conjugators, a majority of about three quarters the over 200 subjects investigated which possess the enzyme, and 3) nonconjugators, about one quarter of the over 200 subjects investigated which lack this enzyme in erythrocytes. The DCM metabolism in hemolysate can be described by Michaelis-Menten kinetics; for an individual with high GSTT1 enzyme activity, the maximum velocity V_{max} of formaldehyde production was calculated to be approximately 180 picomole per minute per mg hemoglobin (Hb); the Michaelis constant K_m was approximately 60 mM DCM (Hallier et al. 1993, 1994). A cDNA that encodes a human GSTT1 was found to share 82 percent sequence identity with rat GST5-5. GSTT1 appears to be absent from 38 percent of the investigated population, and the presence and absence of the GSTT1 gene is coincident with the conjugator and nonconjugator phenotypes, respectively (Pemble et al. 1994).

DCM was used to characterize the genetic polymorphism of the human GSTT1 among 140 healthy German volunteers. The ethnic differences in the prevalence of the homozygous deleted genotype exist even between closely related German and Swedish populations (Bruhn et al. 1998). The hereditary transmission of the three human GSTT1-1 conjugator phenotypes in a German family was evaluated. Twenty-nine members of three generations were phenotyped and genotyped with respect to human GSTT1-1. The enzyme activity of high conjugators was twice as high as that of low conjugators. The distribution of GSTT1-1 phenotypes indicated a Mendelian intermediary inheritance, in which a gene-dosage effect resulted in a doubled enzyme expression in the presence of two functional alleles. This Mendelian intermediary inheritance was further supported by the findings of a semiquantitative polymerase chain reaction method

designed to distinguish the three phenotypes of human GSTT1-1 for rapid screening of large study groups (Wiebel et al. 1999).

Interindividual differences in the *in vitro* conjugation of DCM with glutathione by cytosolic GST were investigated with 22 human liver samples. In three of the samples, no activity towards DCM was observed. Eleven samples showed an activity ranging from 0.2 to 0.41 nmole/minute/mg protein with an average of 0.31 ± 0.08 nmole/minute/mg protein. Eight samples showed an activity ranging from 0.82 to 1.23 nmole/minute/mg protein with an average of 1.03 ± 0.14 nmole/minute/mg protein. The highest activity towards DCM was 1.4 times lower than, or only 70 percent of the activity in rat cytosol (Bogaards et al. 1993).

Immunohistochemical localization of GSTT1 in mouse kidney, liver, and lung was reported using a novel antibody recognizing mouse and rat but not human GSTT1 (Quondamatteo et al. 1998). Species differences in GSTT1-1 activity towards DCM in liver and kidney were reported. In rats, mice, and hamsters, GSTT1-1 activity in liver cytosol towards DCM was two to seven-times higher than in the kidney cytosol. In humans this activity was twice as high in kidney cytosol as in liver cytosol. The relation between species was mouse > rat > human high conjugator > human low conjugator > hamster > human nonconjugator for liver cytosol, and mouse > human high conjugator > human low conjugator = rat > hamster = human nonconjugator for kidney cytosol (Thier et al. 1998).

Mixed Function Oxidases (MFO)

The MFO pathway metabolizes DCM via an oxidative dehalogenation with microsomal enzymes, primarily the cytochrome P450 isozyme 2E1 (CYP2E1). In the presence of molecular oxygen, nicotinamide adenine dinucleotide phosphate (NADP) and reduced NADP (NADPH), the microsomal MFO metabolize DCM to CO. The sequence of steps in the metabolic transformation of DCM includes hydroxylation and dehalogenation of DCM to formyl chloride, which spontaneously decomposes to CO and chloride ion. It has been postulated that the MFO pathway contributes to the carcinogenicity of DCM through the production of the reactive formyl chloride (OSHA 1997).

The mechanism of MFO-mediated metabolism of DCM described above was developed by Anders and co-workers (Ahmed and Anders 1978, Anders et al. 1977, Kubic and Anders 1975, Stevens and Anders 1978, 1979). Data from these studies showed that the oxygen present in CO formed by the metabolism of DCM was derived from molecular oxygen (O_2) rather than water, suggesting a sequence of metabolism that begins with the hydroxylation of DCM.

The metabolism of DCM to CO was mediated solely by the MFO pathway, and CO can be detected in the blood and breath of humans and animals exposed to DCM. Therefore, CO has been used as a surrogate measure of DCM exposure in humans. CO production has also been used as an index of MFO activity, and thus, as an indicator of the relative contribution of each pathway to the total metabolism of DCM (OSHA 1997).

Various studies have shown that CO_2 is produced from DCM by both the MFO- and GST-mediated metabolism of DCM (Andersen et al. 1987a, Gargas et al. 1986, Green et al. 1987a, Reitz et al. 1986, 1988, 1989). In-depth analyses of the kinetics involved in the metabolism of DCM by specific pathways are discussed elsewhere (ATSDR 1993, Bogen et al. 1992, OSHA 1997, U.S. EPA 1987a).

Overall, saturation of the MFO pathway occurred at similar levels in both mice and rats, but significantly more DCM was metabolized by the GST pathway in the mice when assessed either from the blood DCM levels or by CO_2 formation at high dose levels (ATSDR 1993).

Excretion

In humans, DCM is removed from the body primarily in expired air and urine after inhalation exposures (DiVincenzo et al. 1971). In humans exposed to 100 or 200 ppm DCM for two hours, an average of 22.6 μg (0.003 percent) and 81.5 μg (0.006 percent) DCM was excreted in the urine within 24 hours after exposure (DiVincenzo et al. 1972). Concentrations in expired breath following exposure were about 20 ppm and dropped to about five ppm at the end of 30 minutes (DiVincenzo and Kaplan 1981b). In humans, between 25 and 34 percent of the DCM inhaled was excreted as CO in the expired air, and less than five percent was eliminated unchanged (DiVincenzo and Kaplan 1981a). Urinary formic acid concentrations increased in workers exposed to DCM (Sullivan et al. 1993). No studies were located regarding excretion in humans after oral exposure to DCM.

Forty-eight hours after exposure of rats to DCM, either orally with one or 50 mg/kg or by inhalation with 50, 500, or 1,500 ppm, less than 10 percent or 7 to 23 percent, respectively, of the body burden was retained. Expired air accounted for most of the excreted DCM in rats following either inhalation or oral exposures. Expired air accounted for 58 to 79 percent of the excreted DCM through a single six-hour exposure to 50, 500, or 1,500 ppm DCM; 78 to 90 percent through one or 50 mg/kg by gavage (McKenna et al. 1982, McKenna and Zempel 1981). Excretion via the urine accounts for two to nine percent of the dose (ATSDR 1993).

Physiologically-Based Pharmacokinetics (PBPK) Models

Descriptions of PBPK Models

This section provides brief descriptions of several PBPK models that have been developed to estimate tissue-specific exposures to DCM, taking into account its absorption, distribution, and metabolism in humans and animals (Anderson et al. 1983, Bailer and Dankovic 1997, Blancato 1991, Clewell and Andersen 1989, 1994; Clewell et al. 1994, 1997; Krewski et al. 1994, Rhomberg 1995, Thorslund et al. 1987, U.S. EPA 1987b). More detailed descriptions of these models are provided by the model authors. PBPK models have been used to study the mechanisms of disposition of xenobiotics within the body for about 70 years. In the mid 1980s, quantitative use of PBPK models in DCM carcinogen risk assessment was related to the ability of the models to make more accurate predictions of target dose for different exposure situations in different animal species including humans (Andersen 1995a, 1995b, Andersen et al. 1987a, Conolly et al. 1992). The principal application of PBPK models is in the prediction of tissue dosimetry of the toxic moiety, e.g., parent chemical, reactive metabolites, macromolecular adducts, etc. (Andersen and Krishnan 1994).

Angelo et al. (1984, 1986a, 1986b) and Angelo and Pritchard (1984) developed a PBPK model which characterized the distribution, metabolism, and elimination of DCM following oral administration in mice and rats. The model consisted of seven compartments: lungs, liver, carcass, kidneys, gastrointestinal tract, arterial blood, and venous blood. In this model, DCM was assumed to be cleared from the lungs in a linear fashion, and all metabolic conversion of DCM was assumed to take place in the liver by a single, saturable metabolic pathway. The model predicted DCM concentrations in liver and compared it with experimental data from mice given a daily gavage dose of 1,000 mg DCM in corn oil per kg for 14 days. Comparisons were also made

between *in vivo* and model-simulated pulmonary elimination of DCM, and the metabolites CO and CO_2. Although model predictions of DCM elimination were within a few percent of measured values, it underestimated the percentage of administered dose eliminated as CO or CO_2.

Andersen (1995a, 1995b) and co-workers (1984, 1987a, 1987b, 1991, 1993, 1994) developed a PBPK model to provide quantitative estimates of the levels of DCM in various organs of four mammalian species, rats, mice, hamsters, and humans, following inhalation exposure. The model consists of compartments including the exposure chamber, lung, liver, fat, and richly perfused and slowly perfused tissues. The model, which incorporates a variety of variables representing the blood and tissue concentrations of DCM, exhaled DCM, and instantaneous rates of metabolism by each pathway, was validated by comparing predictions of DCM blood concentration and time-course data with experimentally derived results obtained with Fischer 344 rats, Syrian Golden hamsters, B6C3F1 mice, and human volunteers.

Body tissues were separated into five compartments of lung, liver, fat, richly perfused, and slowly perfused on the basis of similar blood flows and partition coefficients. In this model, the metabolism of DCM was assumed to take place in the liver and lung, mediated by two separate enzyme systems, the MFO- and the GST-mediated pathways. Metabolism of DCM by MFO was assumed to be saturable and governed by the two Michaelis-Menten parameters, maximum velocity V_{max} and Michaelis constant K_m, while GST-dependent metabolism was modeled as a first order linear kinetic process governed by the single rate constant, K_F. Inhalation exposures to DCM were modeled on the assumption that gas to blood exchange occurs before DCM enters the metabolically active lung compartment. For drinking water simulations, DCM was assumed to be completely absorbed by the gastrointestinal tract, and introduced into the liver compartment at a constant hourly rate over the entire exposure period.

Blood to air partition coefficients were measured in blood samples from all four species - rat, hamster, mouse, and human. Tissue to air partition coefficients were determined for liver, fat, and muscle using tissue homogenate in rats and hamsters. The coefficients for the lung and richly perfused tissue were assumed to be equal to the values obtained for liver. Partition coefficients for various tissues were not measured for mice or humans, but were assumed to be equal to those measured in rats. Estimates of the parameters V_{max}, K_m, and K_F governing overall DCM metabolism were obtained by mathematical optimization of the PBPK model to data based on the decline in DCM concentration in exposed B6C3F1 mice and Fischer 344 rats. For humans, V_{max} and K_m for the MFO pathway were obtained from experimental data (Andersen et al. 1991) on levels of DCM and COHb in human blood and DCM and CO in expired air after exposure of volunteers to a known concentration of DCM. Those parameters for the GST-mediated pathway were calculated by allometric scaling of the corresponding rodent data (Andersen et al. 1991).

The model was used to calculate six different measures of target tissue dose based on the applied doses of DCM used in the bioassays of DCM conducted by the NTP (1986) and Serota et al. (1986a, 1986b). The NTP (1986) study found significantly elevated increases in liver and lung tumors in mice exposed to 2,000 or 4,000 ppm DCM by inhalation, while Serota et al. (1986b) found limited evidence of carcinogenicity in mice given up to 250 mg/kg-day of DCM in drinking water. A numerical technique with DCM suggested that incorporating intrapopulation variability of biologically interpretable parameters to the model could increase the variability of safe dose estimates (Portier 1987, Portier and Kaplan 1989).

Andersen et al. (1987a) found that the model predicted widely different target tissue doses of GST-derived metabolites for the two bioassay exposure scenarios. Depending on whether the

target tissue was the liver or the lung, inhalation of DCM at the exposure concentrations listed above resulted in target tissue doses that ranged from 56 to 256 times that received by animals

given DCM in drinking water. By comparison, model-generated estimates of target tissue doses of MFO-derived metabolites were similar for both exposure protocols. The target tissue doses calculated for liver following inhalation of DCM at 2,000 or 4,000 ppm (NTP 1986 study) were 69 and 71 percent, respectively, of those calculated for DCM administered in drinking water (Serota et al. 1986b study). For the lung the values were 125 to 129 percent of those calculated for DCM administered in drinking water. From these data, Andersen et al. (1987a) concluded that it is unlikely that the carcinogenicity of DCM is due to a metabolite produced by the MFO pathway, but rather, that carcinogenicity may be related to the production of a reactive metabolite by GST, or to the concentration of DCM itself in target organs.

One of the criticisms of the Andersen et al. (1987a) study has been that the pathway-specific values of V_{max} and K_m in rodents were based on measurements of enzyme specific activity made with substrates other than DCM (U.S. EPA 1987a, 1987b). An additional limitation was that the kinetic constants for GST metabolism of DCM in humans were estimated by allometric scaling rather than from experimental data. To address these concerns, Reitz (1991b) and his colleagues (1988, 1989) updated the PBPK model of Andersen et al. (1987a, 1987b) using *in vitro* data. The *in vitro* specific activity of GST- and MFO-mediated metabolism of DCM in lung and liver samples from mice, rats, hamsters, and humans was measured (Reitz 1991b, Reitz et al. 1988, 1989). However, values for V_{max} and K_m for GST and MFO metabolism of DCM in human and mouse liver were determined by numerical optimization of a model fit to the *in vitro* data. A similar estimation procedure for rat and hamster liver samples was unsuccessful in that no "reasonable" solution was found. The studies of Reitz (1991b) and his colleagues (1988, 1989) are limited by the use of these optimization procedures to obtain various estimates of V_{max} and K_m which may substantially influence the final estimates of internal or "tissue" dose of DCM.

Andersen et al. (1991) adapted their earlier PBPK model to describe and predict oxidative DCM metabolism to CO and COHb in rats and humans. Physiological and biochemical constants for the CO to COHb portion of the combined model were estimated from data obtained from male Fischer 344 rats exposed to 200 ppm DCM for two hours. The human physiochemical parameters for COHb and CO were obtained by allometrically scaling those obtained for rats. The corresponding human model was found to provide reasonably good data from two earlier studies involving human respiratory exposures to 50, 100, 250 or 500 ppm DCM for 7.5 hours (Peterson 1978) and to 986 ppm DCM for two hours (Stewart et al. 1972a). Andersen et al. (1991) did not use their human PBPK model to estimate rates of human DCM metabolism or compare such rates with those predicted by the models of Andersen et al. (1987a) or Reitz et al. (1988, 1989). However, given the overall similarity of the model structures and parameter values, any differences in total or pathway-specific rates of DCM metabolism predicted by these models are expected to be less than one order of magnitude.

A modified PBPK model was developed to derive the impact of exercise and intersubject variability on DCM dose estimates (Dankovic and Bailer 1994). The modifications produced increases in the GST pathway doses for the liver and lung varying from 0 to 5.4 and 0 to 3.6 times those computed by the original model, respectively. The authors concluded that some occupationally exposed individuals might receive GST metabolized DCM doses that are several times larger than those originally predicted assuming that the GST pathway is the primary DCM carcinogenicity mechanism. Yoshida et al. (1993) reported that estimated doses of human exposure to DCM using PBPK and environmental fate models were in agreement with measured values in Japan. McDougal et al. (1986) developed a PBPK model with a dermal compartment to provide means for relating laboratory animal exposures to human situations.

OSHA (1997), after examining the PBPK models submitted, concluded that "PBPK modeling estimates of the amount of GST metabolites produced are reasonable dose surrogates for DCM

and are supported by substantial scientific evidence in the record." In its quantitative risk assessment, OSHA (1997) modified an existing model (Clewell 1993, 1995) with a Bayesian analysis to account for the covariance of the PBPK parameters and to incorporate distributions of physiological parameters obtained from the scientific literature.

A recent preliminary study reported the effects of GSTT polymorphism on the risk estimates of DCM to humans (El-Masri et al. 1999). As described earlier in this report, the carcinogenic potential of DCM has been linked to its metabolism to formaldehyde by GSTT1 (Casanova et al. 1997). GSTT1 is polymorphic in humans (Whittington et al. 1999). The frequency of the GSTT1 homozygous null genotype ranges from 10 to 60 percent in different ethnic and racial populations around the world. El-Masri et al. (1999) investigated how varying GSTT1 genotype frequencies would impact cancer risk estimates for DCM by the application of Monte Carlo simulation methods in combination with PBPK models. The PBPK model was used to estimate the DNA-protein cross links caused by metabolism of DCM. Cancer potency of DCM was obtained by the application of the estimated DNA-protein cross-link amounts to the results of a carcinogenicity study by NTP (1986) in B6C3F1 mice. Human risks were estimated based on the carcinogenic potency of DCM to mice and PBPK-predicted amounts of DNA-protein cross-links formed in humans. The Monte Carlo simulations were used to provide distributions of risk estimates for a sample of 1,000 PBPK runs, each run representing a collection of biochemical and physiological parameters for a single person (with and without polymorphism included in the model). The results show that average and median risk estimates were 23 to 30 percent higher when GSTT1 polymorphism was not included at inhalation DCM doses of 1, 10, 100, and 1,000 ppm. This increase in risk was significantly reduced when it was based on the 95^{th} percentile measure for all the doses. The specific effect of this polymorphism on population risk was further investigated by varying the probability that an individual may have a nonfunctional form of the enzyme at a constant dose level of 10 ppm of DCM. Higher values of this probability resulted in a corresponding decrease in risk. Again, this drop in population risk was not as significant when the 95^{th} percentile measure was used (El-Masri et al. 1999).

Previous Applications of PBPK Models for DCM

In the risk assessment of DCM as a TAC and a carcinogen under Proposition 65, risks from ambient exposures using both mechanistic and distribution models were calculated (DHS 1989). Additionally, the Andersen et al. (1987a, 1987b) PBPK model was used to estimate the delivered dose of DCM to the mouse lung. The cancer risk was then estimated for humans using this calculated delivered dosage. As summarized in this report, the risk assessment of DCM in drinking water for the then proposed RPHL of three ppb was based on these calculations from the DHS (1989) document.

Animal Data

For the estimates of cancer risk, DHS (1989) used the data of sarcomas of the salivary gland region in Sprague-Dawley male rats (Burek et al. 1984), alveolar and bronchiolar neoplasms of the lung in B6C3F1 male and female mice (NTP 1986), and hepatocellular adenomas or carcinomas in female B6C3F1 mice (NTP 1986) from DCM animal bioassays. Standard unit risk estimates (q_1* in units per ppb) were obtained for these endpoints using the linearized multistage model.

The lung tumors in mice were used as a basis for a more detailed, quantitative, risk-modeling effort because the tumor data indicated a strong increasing response with dose ($p \leq 0.001$ by

Cochran-Armitage trend test) and were consistent with the multistage hypothesis; the response was consistent between sexes; and the largest 95 percent upper-bound linear coefficient (q_1*) for the four data sets considered was associated with the lung tumor response in female mice. Although the data were from inhalation exposures, it is applicable to oral intake because DCM is absorbed rapidly following either inhalation or oral exposures and the elimination kinetics of DCM were consistent with results generated by both oral or inhalation exposures (U.S. EPA 1991).

The multistage time-dependent and time-independent models were compared to a number of alternative models that have been used in risk assessment. The risk values assumed that humans and animals exposed to equal doses of a carcinogen (mg per surface area) for an equivalent proportion of a lifetime would encounter the same degree of risk. The various models gave comparable results, as indicated by the ratio of the highest to the lowest value for the 95 percent upper-bound estimates of about a factor of two.

In addition to estimates derived under the standard "applied dose" approach, risk estimates for DCM have been derived using variations of the PBPK model described by Andersen et al. (1987a, 1987b). Staff members of the U.S. EPA (1987a, 1987b) and the U.S. CPSC (1987) have used this approach through the U.S. Interagency Hazard/Risk Assessment Committee of the Integrated Chlorinated Solvents Project (HRAC) and also have independently derived PBPK values for the risk assessment of DCM. DHS (1989) discussed application of the PBPK model to risk assessment and summarized the values that were used in reporting the range of risks from DCM exposure.

Identification of Metabolites Active in Carcinogenicity

The observed tumorigenicity of DCM appears to be related to the formation of glutathione conjugates via the GST pathway. However, it is unclear how significant a role the GST pathway plays and whether the contribution of the MFO pathway is significant. There is considerable uncertainty with regard to the identification of the DCM-glutathione conjugate as the sole metabolite responsible for carcinogenicity in laboratory animals and with regard to the exclusion of MFO activity from the production of carcinogenic metabolites.

HRAC (U.S. EPA 1987a) indicated that "the mechanism of carcinogenic action of [DCM] remains problematical..." and "...there remains some uncertainty as to whether or not the GST pathway is the sole path to carcinogenicity." The CPSC (1987) document stated "some or all of the intermediates of the various pathways, or the parent compound, may contribute to the carcinogenic process." Although there is uncertainty in positively identifying the carcinogenic species, U.S. EPA and CPSC concluded that the MFO pathway only plays a minor role in the carcinogenic process in animals, and risk estimates were made using the PBPK model assuming that the carcinogenic activity of DCM is not dependent on the MFO pathway. OSHA (1997) made similar conclusions.

An extensive analysis of the PBPK model was presented in the federal interagency HRAC draft report (U.S. EPA 1987a). HRAC suggested that pharmacokinetic model adjustments consisted of two separate analyses. The first analysis adjusts for saturated metabolic pathways during the experimental exposure (referred to as a high-to-low-dose adjustment). The second adjusts for interspecies differences in the handling of the substance (referred to as the species-to-species adjustment). The HRAC analysis concluded that the PBPK model could be used to adjust for saturation of the MFO pathway in calculating the risk estimate for DCM. The U.S. EPA analysis (1987b) went further and suggested that an adjustment for interspecies differences could also be

made using the PBPK model. The analyses discussed below are based on the HRAC report (U.S. EPA 1987a), the CPSC (1987) report, and the U.S. EPA (1987b) report.

Adjustment for MFO Saturation: High-to-Low Dose

Based on data discussed earlier, the MFO pathway is expected to be saturated at all dose levels in mice in the NTP (1986) inhalation bioassay. In the CPSC (1987) analysis, the three metabolic constants in humans in the PBPK model were estimated; K_F which is the first order rate constant for the GST pathway, K_M which is the Michaelis-Menten constant for the MFO pathway, and V_{MAX} which is the maximum velocity of metabolism by MFO. The constant estimates, 0.53, 0.58, and 118.9, for K_F, K_M, and V_{MAX}, respectively, required an adjustment of 2.2 when extrapolating from high to low dose for the mice lung tumor data (CPSC 1987). This adjustment factor was shown to be relatively insensitive, varying from 1.6 to 2.4, to changes in the three metabolic variables. Consequently, consideration of the pharmacokinetic adjustments from high-to-low dose would reduce the estimated lung cancer potency by approximately 2.2 fold.

Species-to-Species Adjustment

The CPSC (1987) did not make a species-to-species adjustment for DCM as "the necessary assumptions may introduce even more uncertainty than the present empirical procedure."

The HRAC (U.S. EPA 1987a) studied two PBPK models. One was the model developed by Andersen and co-workers (Andersen et al. 1984, 1987a, 1987b, 1991, 1993, 1994; Andersen and Krishnan 1994, Andersen 1995) based on inhalation of DCM and the other by Angelo and co-workers (Angelo et al. 1984, Angelo and Pritchard 1984, Angelo et al. 1986a, 1986b) based on intravenous and oral exposure to DCM. The two models differ significantly in structure. The U.S. EPA (1987b) chose to develop risk estimates based on the Andersen et al. (1987a, 1987b) approach and to use the Angelo et al. (1986a, 1986b) model to obtain greater insight into the Andersen et al. PBPK model.

The Andersen et al. model estimates tissue-level doses for DCM and its metabolites. Andersen et al. (1987a) concluded that the estimate of risk from DCM exposure to the human lung was 144-fold lower using the PBPK approach compared to the applied-dose approach. U.S. EPA (1987b) indicated that this difference was composed of an 11.3-fold difference based on the estimated dose adjustment, and a 12.7-fold factor used for surface area correction for dose when extrapolating across species. Andersen et al. (1987a) used a lower resting human breathing rate, 12.5 m^3/day instead of 20 m^3/day, and a higher mouse breathing rate (0.084 m^3/day) than the standard U.S. EPA estimate (0.043 m^3/day).

Andersen and Krishnan (1994), using the B6C3F1 mouse data, estimated a cancer risk to humans of 3.7×10^{-8} for a lifetime inhalation exposure of 1 $\mu g/m^3$, which is lower by more than two orders of magnitude than that calculated by the U. S. EPA using the linearized multistage (LMS) model for low-dose extrapolation and body surface area correction factor for interspecies scaling.

Using the PBPK model developed by Andersen et al. (1987a), U.S. EPA (1987a) calculated the average daily concentration of DCM metabolites in the lung via the GST pathway for mice exposed to 2,000 or 4,000 ppm DCM based on the NTP (1986) bioassay. The internal dose calculated for humans in the lung from exposure at 1 ppm was 0.008386 mg/L/day. The unit risk reported by HRAC (U.S. EPA 1987a) was 1×10^{-6} $(ppb)^{-1}$, or 3×10^{-7} $(\mu g/m^3)^{-1}$. The risks from DCM calculated from the female mouse lung tumor data using various assumptions were compared. Approximately a nine-fold reduction in estimated risk was achieved by the combined

high-to-low dose and species-to-species adjustments. This reduction was mostly due to the assumptions concerning the species-to-species adjustment.

Surface Area Correction Factor

The HRAC (U.S. EPA 1987a) analysis differed from the approach suggested by Andersen et al. (1987) regarding the surface area correction on applied dose. Andersen et al. (1987) assumed that the adjustment for metabolic differences across species in the PBPK model should replace the surface area adjustment (U.S. EPA 1987a). The HRAC concluded that the surface area conversion factor (12.7) is still needed to account for interspecies differences in sensitivity. However, the CPSC analysis of Andersen's PBPK model indicated that the surface area adjustment has been used to account for the many differences between species, e.g., metabolic activation rates, clearance, distribution, cell sensitivity, efficiency of DNA repair, and cell proliferation rates.

There are likely to be differences in tissue susceptibility within a species. Reitz et al. (1988) reported the PBPK internal dose estimates for lung and liver for the NTP (1986) mouse bioassay. Despite a higher PBPK dose delivered to the liver than to the lung, the tumor response is slightly lower in the liver than the lung. The lung was found to be approximately nine-fold more sensitive to the PBPK dose than the liver. Thus, for the mouse there was a substantial difference in tissue susceptibility to the PBPK dose. Consequently, the PBPK dose should not be considered the sole factor affecting intraspecies or interspecies differences. There may also be differences in susceptibility across species to the PBPK dose. At present an estimate of the difference has not been made using published PBPK doses.

Based on the nine-fold difference between two tissues in the mouse NTP (1986) study, there are likely to be differences in susceptibility across species. DHS (1989) agreed with U.S. EPA (1987a) assessment that the application of the surface area correction to account for sensitivity differences was justified, in light of the numerous uncertainties involved in the PBPK procedure. Furthermore, DHS (1989) believes that the surface area correction is needed to account for the potential interspecies differences in response to DCM exposure. OEHHA staff concurred with DHS (1989) and U.S. EPA (1987a) in this approach.

Previously Proposed Recommended Public Health Level (RPHL)

Various approaches were used to develop models to obtain the potential theoretical increase in cancer risk associated with continuous lifetime exposure to DCM in ambient air (DHS 1989). The risks estimated for lifetime exposure to 1 ppb DCM, based on induction of lung tumors, range from 1×10^{-6} to 10×10^{-6} (0.3 to 3×10^{-6} from exposure to 1 $\mu g/m^3$). The PBPK-adjusted values required parameter estimates from a model as reported by HRAC (U.S. EPA 1987a), U.S. EPA (1987b) and the CPSC (1987). The lower estimate (1×10^{-6} per ppb) incorporates a complete pharmacokinetic adjustment as calculated by U.S. EPA (1987b). DHS (1989), in evaluating the various approaches, believed that the complete pharmacokinetic adjustment retains considerable uncertainty. In contrast, the applied dose value (10×10^{-6} per ppb) does not incorporate any of the available pharmacokinetic information, and thus ignores information regarding saturation of the MFO pathway in the NTP study; the likely result is that the applied dose value overestimates the risk. The high-to-low dose adjustment used by CPSC to generate a risk of 4×10^{-6} per ppb incorporates information regarding saturation of the MFO pathway in the NTP study. However, the high-to-low dose adjustment assumes a negligible contribution by the MFO pathway to the cancer risk of DCM, and thus may underestimate the risk. DHS (1989)

concluded that the full range of values presented above is scientifically plausible; however, after evaluation of the HRAC data (U.S. EPA 1987a) and other available evidence, the CPSC (1987) adjusted value of 4×10^{-6} per ppb appeared to be the most likely estimate, available through literature, of the risk of DCM exposure.

DHS (1989) then calculated an estimate using the two-year NTP (1986) mouse inhalation study for the determination of risk values for DCM. Cancer potency values near 1×10^{-6} per ppb were recommended as the "most likely estimate of the upper limit of risk" per unit exposure (DHS 1989). This is equivalent to 0.001 to 0.01 $(mg/kg\text{-}day)^{-1}$, with the best estimate of the upper limit on risk equal to 0.0035 or 3.5×10^{-3} $(mg/kg\text{-}day)^{-1}$. The calculated oral cancer potency was 3.5×10^{-3} $(mg/kg\text{-}day)^{-1}$.

In evaluating the PAD factors for water-based exposures, three pathways including water ingestion, inhalation, and skin absorption, are considered. These PAD factors were used to obtain "best estimate" and "upper bound values." As explained later in the exposure assessment section, OEHHA considers this "best estimate" of 6 L_{eq}/day to be the most appropriate to be used for the derivation of the public health-protective concentration for DCM. Using a default adult male body weight of 70 kg, a default *de minimis* lifetime excess individual cancer risk of 10^{-6}, the previously proposed RPHL as a public health-protective concentration accounting for carcinogenic effects of DCM was calculated to be 3.34×10^{-3} mg/L, or rounded to 3 ppb.

Concerns Regarding the PBPK Model

While the general approach and structure of the PBPK model is attractive and seems reasonable, several issues regarding the ultimate accuracy of the PBPK model over the concentrations of DCM metabolites of interest remain unresolved. These issues are:

- More information is needed to validate the predicted results of the PBPK model. The effects of some variations in model design on the final results need to be quantified.
- Further research is needed to elucidate the relative importance of the DCM glutathione conjugates and the MFO pathway in human carcinogenesis. The effects of the GST polymorphism on the risk estimates of DCM to human populations need further evaluation. Lack of quantitative knowledge of the issues of polymorphism, and age specific differences including those associated with early in life and in utero exposures requires further studies.
- Due to the large number of variables and equations used in the PBPK models, the sensitivity of the models to changes in these parameter estimates should be further evaluated.
- Sites other than the lung and liver should be assessed in terms of their contribution to metabolism and their susceptibility to carcinogenicity.

On the other hand, the PBPK approach does allow for consideration of pertinent data that cannot be taken into account in the applied dose approach. Thus, despite the concerns enumerated above, cautious application of the PBPK model to DCM risk assessment is appropriate in conjunction with other more traditional approaches.

TOXICOLOGY

The toxicology profile of DCM has been summarized in the U.S. by ATSDR (1993) and Dhillon and Von Burg (1995), by European organizations (ECETOC 1984, OECD 1994) and by the IPCS (1996) under WHO. Health risk assessment of DCM has been performed (Bogen et al. 1992,

DHS 1989, U.S. EPA 1992a, OSHA 1997) and the general toxicity of DCM is not considered as "highly hazardous" (HSDB 1999). OSHA (1997) and IARC (1999) summarize the exposure to DCM and human health effects including ten epidemiological studies and seven animal carcinogenesis studies. However, most of the studies and reviews focus on the inhalation route of exposure in human health effects and laboratory animal toxicities. No studies were located regarding toxic effects in humans after oral exposure to DCM alone. Because this document is mainly concerned with the effects of DCM in drinking water, it focuses on oral toxicity studies in animals. There is limited information on dermal exposure effects in humans and animals. Very little is known about the toxic effects of DCM in plants and ecosystems.

Toxicological Effects in Animals and Plants

The adverse effects of DCM in animals following acute, subchronic, and chronic exposure have been reviewed by the ATSDR (1987, 1993), ACGIH (1990, 1995), Brandt and Okamoto (1988), DHS (1989), Dhillon and Von Burg (1995), Environment Canada (1993), ECETOC (1987), IARC (1986, 1999), OEHHA (1992), OSHA (1997), U.S. EPA (1984, 1985a, 1985b), Strobel and Grummt (1987), as well as summarized in various databases in TOMES PLUS® system (Hall and Rumack 1999). Table 3 presents a summary of the noncarcinogenic adverse effects of DCM on its principal target organs and systems in humans and animals. The carcinogenicity of DCM is discussed in detail later in this report.

DCM exhibits low acute toxicity in experimental animals. The 50 percent lethal concentration (LC_{50}) values were between 12,000 and 18,000 ppm at four to seven hours of exposure in rats, mice, and guinea pigs. An oral 50 percent lethal dose (LD_{50}) of 2,100 mg/kg was reported in rats. CNS toxicity, characterized by lethargy, incoordination, narcosis, restlessness, and altered behavior, is the predominant effect associated with acute DCM exposures in experimental animals. Metabolic conversion of DCM to CO may produce reversible CO intoxication and neurotoxicity. Inhalation exposure to approximately 1,000 to 20,000 ppm DCM can lead to loss of the righting reflex and death in animals, with species differences in sensitivity.

As detailed later in this document, other important acute and subchronic toxic effects reported include liver damage consisting of increased liver triglycerides and serum bilirubin, fatty infiltration, and necrosis of hepatocytes; cardiotoxicity consisting of cardiac arrhythmia; hematopoietic disturbance in blood pressure; pulmonary irritation and foreign body pneumonia; and irritation of the eyes.

Testicular atrophy was observed in rats and mice exposed to the high concentration of 4,000 ppm of DCM for two years, but no adverse effects on the reproductive system were observed at lower levels. Animal studies showed that DCM can cross the placental barrier. No treatment-related visceral abnormalities were seen in exposed fetuses of mice or rats but minor skeletal deformities or incomplete ossification and decreased fetal weight gain were reported in both species.

In terms of genotoxicity, *in vitro* tests in bacteria and in mammalian cells gave positive results in most of the tests that indicated a mutagenic potential of DCM. Chromosomal aberrations were seen in some studies, but not in others. Given the evidence of *in vitro* clastogenicity and its negative results in unscheduled DNA synthesis (UDS) and DNA binding studies, DCM may be a mutagen *in vitro* in mammalian systems. *In vivo* tests to assess its potential to induce gene mutation, chromosomal aberrations or DNA damage or repairs produced some positive but also many negative results.

Animal studies showed that DCM is carcinogenic to rodents by inhalation and ingestion. DCM administered by inhalation increased the incidence of alveolar and bronchiolar neoplasms and

hepatocellular carcinomas and adenomas in mice of both sexes. In female and male rats, DCM produced dose-related increases in mammary tumors and increases in the number of tumors per tumor-bearing rat. In a drinking water study, the incidence of liver tumors significantly increased over concurrent control levels in female rats. In the same drinking water study, male mice had an elevated incidence of combined hepatocellular carcinomas and neoplastic nodules.

Table 3. Significant Noncancer Health Effects and Levels of Oral Exposure to Dichloromethane in Animals†

Species/ (Strain)	Exposure/ Duration/ Frequency (Specific route)	System	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
ACUTE EXPOSURE					
Death					
Mouse	Oral			1,987 (LD_{50})	Dhillon and Von Burg 1995
Rat	Oral			1,600 (LD_{50})	Dhillon and Von Burg 1995
Rat	Once a day (gavage in water)			2,100 (LD_{50})	Kimura et al. 1971
Rat	Once a day (gavage in oil)			4,382 (LD_{95})	Ugazio et al. 1973
SUBCHRONIC EXPOSURE					
Systemic Toxicity					
Rat (Fischer)	90 days (ingestion in drinking water)	Hepatic	166 (male) 209 (female)	209 (hepato-cellular vacuolization, altered clinical chemistry) 1,200 (centrilobular necrosis)	Kirsch-man et al. 1986
		Renal	1,200		
Mouse (B6C3F1)	90 days (ingestion in drinking water)	Hepatic	226 (male) 231 (female)	587 (centri-lobular fatty changes)	Kirsch-man et al. 1986
Rat (Wistar)	13 weeks (ingestion in drinking water)	Hepatic	15 (the only dose tested)		Born-mann and Loeser 1967

Table 3 (Continued). Significant Noncancer Health Effects and Levels of Oral Exposure to Dichloromethane in Animals†

Species/ (Strain)	Exposure/ Duration/ Frequency (Specific route)	System	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
CHRONIC EXPOSURE					
Systemic Toxicity					
Rat (Fischer)	78 – 104 weeks (ingestion in drinking water)	Hepatic	6	50 (increased foci of cellular alteration and fatty changes)	Serota et al. 1986a
		Hematological	6		
Mouse (B6C3F1)	104 weeks (ingestion in drinking water)	Hepatic	185	250 (increase in fat content)	Serota et al. 1986b
		Hematological	6		

†adapted from ATSDR (1993) and U.S. EPA (1984, 1985a, 1985b, 1987a, 1987b, 1992a, 1999)

Acute Toxicity

Acute exposure to DCM causes skin, eye, and respiratory tract irritation and burns. DCM vapors cause incoordination in respiration and lethargy. When DCM was instilled into the conjunctival sac of rabbits, inflammation of the conjunctiva and eyelids, keratitis, iritis, and increased corneal thickness and intraocular tension were observed. These effects were reversible within three to nine days after the treatment. Increases in the thickness of the cornea in rabbits exposed to DCM vapor at about 490 ppm, equivalent to about 1,750 mg/m^3, for 10 minutes were also reported, but the effect was reversible in two days (Ballantyne et al. 1976).

An oral LD_{50} value of 2,100 mg/kg was reported in rats that ingested DCM in drinking water for one time in one day (Kimura et al. 1971). An oral LD_{50} in Sprague-Dawley rats was found to be 1.72 mL/kg or about 2,282 mg/kg for males, and 1.06 mL/kg or about 1,406 mg/kg for females (Laham et al. 1978). Acute exposure to DCM at 1,095 mg/kg-day by gavage resulted in CNS depression, vascular disturbance, liver necrosis and gastrointestinal hemorrhage in rats. Ninety-five percent lethality (LD_{95}) was reported in rats gavaged with 4,382 mg/kg DCM in oil for one time in one day (Ugazio et al. 1973). The acute oral LD_{50} in rats was reported to range from 2,100 to 3,000 mg/kg. The subcutaneous LD_{50} in mice was approximately 6,400 mg/kg. The intraperitoneal LD_{50} in mice and dogs was 2,000 and 1,300 mg/kg, respectively (IARC 1986, IPCS 1984, 1996, Sullivan et al. 1993).

Dhillon and Von Burg (1995) reviewed and summarized an oral LD_{50} of 1,600 mg/kg in rat, an oral LD_{50} of 1,987 mg/kg in mouse, an intraperitoneal LD_{50} of 1,500 mg/kg in mouse, a 15-minute inhalation LC_{50} of 2,000,000 mg/m^3 in rat, an eight-hour inhalation LC_{50} of 56,176 mg/m^3 in mouse, and a six-hour inhalation LC_{50} of 40,252 mg/m^3 in guinea pig. An approximate lethal dose of DCM given via the intratracheal route to male Sprague-Dawley rats was 0.35 g/kg or 350 mg/kg, about 17.5 percent of the reported oral LD_{50} of 2,000 mg/kg (McCarty et al. 1992).

The LC_{50} values for male and female rats exposed for six hours to DCM vapors through inhalation were approximately 16,000 to 18,100 ppm (Berger and Fodor 1968, Laham et al. 1978). The LC_{50} value for guinea pigs inhaling DCM for one day was about 11,500 ppm (Balmer et al. 1976). The LC_{50} values for mice exposed for four, six and seven hours were 17,703 ppm (NTP 1986), 14,100 ppm (Gradiski et al. 1974) and 16,189 ppm (Svirbely et al. 1974), respectively. The LC_{50} values for rats exposed for six to seven hours was about 16,000 ppm (approximately 56,480 mg/m^3). A 30-minute LC_{50} for rats was reported to be approximately 25,400 ppm. Exposure to 16,000 to 19,000 ppm DCM by inhalation for four to eight hours caused death in rats and mice (ATSDR 1993). Seventy percent of the mice exposed to 17,250 ppm DCM for four hours died but no death was observed at 16,800 ppm (NTP 1986). Concentrations in excess of 50,000 ppm are thought to be immediately life threatening (Hall and Rumack 1999).

Studies involving short-term exposures of experimental animals to DCM vapors indicate that the CNS is the primary target organ (Savolainen et al. 1981). Decreased rapid eye movement (REM) in sleep and increased sleeping time were observed in albino rats at levels above 500 ppm for 13 hours (Berger and Fodor 1968). Sleep disturbance and reduction in spontaneous activity were reported after exposure to a near lethal dose. The production of COHb in blood in rats, mice, and rabbits after acute exposure to DCM induced the same neurological toxicity as CO poisoning (Roth et al. 1975). OSHA (1997) summarized animal data to show that limited amounts of DCM exposure could cause specific changes in the neurological system at a biochemical level. Alexeeff and Kilgore (1983) showed that learning ability was impaired in mice one to four days after a single oral gavage exposure to 168 mg/L DCM (approximately 40,000 ppm). Interaction of DCM and ethanol in rats reduced nerve conduction velocity (Glatzel et al. 1987). DCM

produced CNS depression in rats following acute oral doses, and cumulative toxicity as well as lethality after repeated dosing within 14 days (Moser et al. 1995).

Exposures to DCM vapor produced irritation of the eyes, skin, and respiratory system in rabbits (Duprat et al. 1976). Exposures to high levels of DCM vapor also produced adverse effects on the cardiovascular system, liver and kidney. Cardiovascular changes in rabbits and dogs in cardiac output, stroke volume and cardiac arrhythmias were reported following short-term exposures of about five minutes or longer to concentrations of DCM exceeding 5,000 ppm (Aviado 1975, Aviado et al. 1977, Taylor et al. 1976, Zakhari 1977). Cardiac arrhythmia was also reported in mice following exposure to DCM (Aviado and Belej 1974). Depression of calcium dynamics in Sprague-Dawley and Wistar rats and in cultured neonatal cardiac myocytes was reported as a common mechanism of DCM cardiotoxicity (Hoffmann et al. 1994, 1995, 1996). Blood pressure reduction by DCM was reported in studies in spontaneously hypertensive rats (Douglas et al. 1976, 1980, Wilkinson et al. 1977).

Female Fischer 344 rats gavaged with DCM at 337 mg/kg-day or higher doses for up to 14 days had increased necrosis of centrilobular hepatocytes (Berman et al. 1995). Weinstein et al. (1972) found that a 24-hour inhalation exposure to approximately 4,900 ppm DCM caused an increase in triglycerides, a decrease in glycogen, and inhibition of protein synthesis in the livers of female mice. Histopathological changes included fatty infiltration, a decrease in the size of centrilobular hepatocyte nuclei, and deterioration of the endoplasmic reticulum. Hepatic effects of increased liver triglycerides, changes in liver weight, and fatty infiltration have been reported in guinea pigs (Morris et al. 1979) following continuous inhalation exposure to approximately 5,000 ppm for six hours.

Pulmonary reactions to acute inhalation of DCM included lipid peroxidation in the lungs in rats (Sahu and Lowther 1980, 1981). Acute bronchiolar Clara cell vacuolation and necrosis after a single exposure to DCM were observed in 12 week old male B6C3F1 mice exposed to 4,000 ppm for six hours. This lesion appeared to correlate with cytochrome P450 activities in whole lung as well as in isolated Clara cells (Foster et al. 1992). Toxic effects on liver and lung of rats and mice following 10-day inhalation exposure were reported (Hext et al. 1986).

The reaction product of DCM with glutathione appears to be the causative agent for acute toxicity in rats and mice based on research on methyl chloride, a gas related to DCM. Acute exposure of rats and mice to methyl chloride caused significant reductions in glutathione levels in organs including liver, kidney, and brain (Dodd et al. 1982). Chellman et al. (1986) showed that depletion of glutathione with glutathione inhibitors diminished acute liver and kidney toxicity as well as CNS and brain toxicity of methyl chloride. Pretreatment with glutathione inhibitors also increased the methyl chloride LC_{50} from 2,200 to 3,200 ppm in male B6C3F1 mice. The lethal effects of a single six-hour inhalation exposure of male mice to 2,500 ppm methyl chloride were completely prevented by pretreatment with a glutathione synthesis inhibitor.

Single intraperitoneal injection of DCM at 300 and 1,000 mg/kg in rats caused changes in lipoproteins in plasma and liver reaching a peak at eight or 19 hours following the administration (Honma and Suda 1997). Acute intraperitoneal injection of DCM in corn oil at 57.5, 115, 230, and 460 mg/kg produced a relative reduction in the amplitude of peak N30 of flash evoked brain-wave potentials in rats with a median effective dose (ED_{50}) of 326.3 mg/kg (Herr and Boyes 1997).

Subchronic Toxicity

Kirschman et al. (1986) administered DCM in drinking water to Fischer 344 rats for 90 days through ingestion at target concentrations of 0.15, 0.45, or 1.5 percent, equivalent to ranges from 166 to 1,469 mg/kg-day. A dose-related increase in the incidence of hepatocellular vacuolization with lipid accumulation and altered clinical chemistry were observed at 209 mg/kg-day and higher doses in males. The same liver effects were observed in females at doses higher than 209 mg/kg-day. Centrilobular liver necrosis, granulomatous foci, and some evidence of ceroid and lipofuscin accumulation were observed at 1,200 mg/kg-day and higher doses, particularly in females. ATSDR (1993) considered that the LOAEL was 166 mg/kg-day for liver toxicity. The U.S. EPA (1992a) considered the NOAEL for male and female rats for liver toxicity as 166 and 209 mg/kg-day, respectively. Kidney weights in exposed rats were elevated without treatment related histopathological lesions; and ATSDR (1993) considered a NOAEL of 1,200 mg/kg-day for renal toxicity. In the same study, B6C3F1 mice exposed to DCM in drinking water were observed with centrilobular fatty changes at 587 mg/kg-day and higher doses. The U.S. EPA (1992a) considered the NOAEL for male and female mice for liver toxicity as 226 and 231 mg/kg-day, respectively.

No adverse effects, except a frequently positive urine albumin test, were reported in male and female Wistar rats after administration of 125 mg/L or 15 mg/kg-day of DCM in drinking water (calculated from an assumed water consumption rate) for 13 weeks (Bornmann and Loeser 1967). The only dose tested, 15 mg/kg-day, was identified as the NOAEL for the study (ATSDR 1993).

Repeated inhalation exposure of subchronic to lifetime duration at levels from 1,000 to 16,000 ppm caused increased deaths in rats, mice, guinea pigs, rabbits, dogs and primates with differences in sensitivity among the species (ATSDR 1993). Death occurred at about 1,000 ppm with dogs, about 5,000 ppm with guinea pigs, and about 10,000 ppm with mice and rats. Dogs and guinea pigs appeared to be more sensitive than mice and rats. Increased mortality was observed in rats exposed to 16,000 ppm DCM by inhalation for 19 days, five days per week, six hours per day (NTP 1986). The highest no-effect level of DCM exposure by inhalation for 90 consecutive days for six hours per day in Beagle dogs was below 5,000 ppm by volume of air or 0.5 percent by volume, and the lowest toxic level in rats was about 10,000 ppm by volume (Leuschner et al. 1984).

Most of the subchronic studies in animals deal with neurobehavioral responses to DCM through inhalation (OSHA 1997). Exposure to 210 ppm or about 741 mg/m^3 in air continuously for three months produced neurochemical changes in neurotransmitter amino acids and enzymes in the brain of Mongolian gerbils (Briving et al. 1986, Karlsson et al. 1987). DCM appears to behave similarly to other anesthetics inducing an initial excitatory phase followed by a progressive depression of the CNS (Savolainen et al. 1977, Thomas et al. 1971, 1972; U.S. EPA 1985a, Weinstein et al. 1972). Exposure to DCM decreases the general level of activity, as well as running and spontaneous activity in mice exposed through inhalation continuously to 5,000 ppm DCM for seven days (Weinstein et al. 1972) or to 1,000 ppm for 14 weeks (Thomas et al. 1972). Slower running activities of male rats inhaling DCM for six hours per day, five days per week, for six months were reported (Heppel and Neal 1944).

Male Wistar rats inhaling 30, 100, or 500 ppm DCM together with formaldehyde or ingestion of aspirin, cadmium chloride, stannous chloride, loperamide, spermine, butyl hydroxyanisol, and di(2-ethylhexyl)phthalate for four weeks had about 10 percent COHb. The authors considered this level of COHb produced by DCM exposure as independent from interactions with the other compounds (Groten et al. 1997). After 13 weeks of inhalation exposure to 50, 200, or 2,000 ppm DCM or 135 ppm CO for six hours a day, five days a week, male and female Fischer 344 rats

exhibited no neurotoxicologic effects with about 10 percent COHb at saturation (Mattsson et al. 1990).

Male B6C3F1 mice aged 12 weeks exposed to 4,000 ppm for six hours per day, five days per week, up to 13 weeks, exhibited on-and-off bronchiolar Clara cell damage. The authors concluded that the lung developed tolerance to DCM with time probably due to inactivation of cytochrome P450 enzymes (Foster et al. 1992). Addition of a cytochrome P450 inhibitor reduced the bronchiolar cell vacuolation seen following exposure to 2,000 ppm DCM. Treatment of mice with a glutathione depletor had no effect on the number of vacuolated bronchiolar cells following DCM exposure. These results suggested that DCM caused Clara cell damage that might be mediated via cytochrome P450 metabolism and the Clara cell vacuolation might have a primary role in stimulating cell proliferation (Foster et al. 1994).

DCM caused moderate centrilobular congestion and degeneration of the liver in female mice exposed to approximately 4,900 ppm for seven days (Weinstein et al. 1972). Similar adverse effects of increased triglyceride levels, centrilobular fat accumulation, and decreased glycogen content were reported in livers of female ICR mice exposed continuously to 100 ppm DCM for up to 10 weeks. Histologic effects in liver were first observed after seven days exposure to DCM at approximately 240 mg/kg-day (Weinstein and Diamond 1972). Haun et al. (1972) observed vacuolization in the livers of rats (estimated to be equivalent to doses of 130 mg/kg-day), dogs (84 mg/kg-day), and monkeys (42 mg/kg-day) exposed to 100 ppm for 100 days. Other hepatic effects reported in rats exposed to 500 to 4,000 ppm were hemosiderosis, focal necrosis of hepatocytes, and cytoplasmic vacuolation (Burek et al. 1984, NTP 1986). At the end of exposure to 300 ppm DCM for 30 days, body weight was significantly decreased and liver weight significantly increased in both sexes of treated NMR1 mice (Kjellstrand et al. 1985).

The NTP (1986) reported inhalation exposure in groups of 10 Fischer 344/N rats and 10 B6C3F1 mice of each sex to five concentrations of DCM at 525, 1,050, 2,100, 4,200 and 8,400 ppm for six hours per day, five days per week, for 13 weeks. In addition to the liver effects mentioned above, this study found that rats developed foreign body pneumonia with foci of mononuclear and multinucleated giant cells, although lung lesions were not reported in mice. Nephrotoxicity was reported in mice exposed to DCM (Plaa and Larson 1965).

DCM was found to be readily absorbed through intact skin in rats exposed for four months through full-body exposure. DCM accumulated in skin and body adipose tissue (McKenna et al. 1982).

Chronic Toxicity

Serota et al. (1986a) exposed male and female Fischer 344 rats for 104 weeks to DCM in the drinking water at concentrations of 0, 5, 50, 125, and 250 mg/kg-day. The numbers of leukocytes and red blood cells and hematocrit and hemoglobin levels increased over concurrent control levels but were within historical control ranges. Statistically significant decreases ($p < 0.05$) in body weight, and in water and food consumption, were observed in rats receiving 125 or 250 mg/kg-day compared with controls. A positive dose-related trend was observed in both sexes for liver histomorphological alterations with foci and area cellular alterations and fatty changes at DCM levels of 50, 125, or 250 mg/kg-day. The hepatic effects were noted at 78 and 104 weeks of the study.

The authors in this study (Serota et al. 1986a) suggested an oral NOEL for these liver effects of 5 mg/kg based on the target dosing level; the level was equivalent to 6 mg/kg-day in both males and females as the estimate of mean daily consumption. ATSDR (1993) identified 6 mg/kg-day as the NOAEL and calculated a chronic oral Minimal Risk Level (MRL) of 0.06 mg/kg-day

based on the observed NOAEL and uncertainty factors totaling 100. U.S. EPA (1999) in its IRIS database identified a NOAEL of 5.85 and 6.47 mg/kg-day for male and female rats, respectively, and used an average of 6 mg/kg-day as the NOAEL to calculate its Reference Dose (RfD).

Serota et al. (1986b) exposed male and female B6C3F1 mice for 104 weeks to DCM in the drinking water at concentrations of 0, 60, 125, 185, and 250 mg/kg-day. An increase in

hepatocellular alterations consisting of increased liver fat content was noted in the high-dose males and females. A NOAEL of 185 mg/kg-day was identified by the authors based on the target level; the level was equivalent to 177 mg/kg-day in males and 172 mg/kg-day in females as the estimate of mean daily consumption.

The NTP (1986) reported that inhalation exposure in Fischer 344/N rats of each sex to concentrations of DCM at 0, 1,000, 2,000, and 4,000 ppm for six hours per day, five days per week, for 102 weeks, resulted in increased incidences of hepatic hemosiderosis, cytomegaly, cytoplasmic vacuolization, necrosis, granulomatous inflammation, and bile duct fibrosis in both males and female rats. In addition, increased incidences of benign mammary tumors were noted in both males and females exposed to 4,000 ppm DCM. NTP also reported that two-year inhalation exposure to 2,000 or 4,000 ppm DCM for six hours per day, five days per week, resulted in hepatic cytologic degeneration in B6C3F1 mice.

Two-year inhalation exposure of Sprague-Dawley rats to 500, 1,500, or 3,500 ppm DCM for six hours per day, five days per week, resulted in hepatic lesions characterized by increased vacuolization in males and females at the 500 ppm level which was equivalent to approximately 165 mg/kg-day (NTP 1986). No effects were noted in Golden Syrian hamsters exposed for two years to 500 to 3,500 ppm DCM for six hours per day and five days per week (Burek et al. 1984).

Toxicity of DCM to fathead minnows (Alexander et al. 1978) and to life stages of the fathead minnow, Pimephales promelas Rafinesque (Dill et al. 1987) was reported. A rapid simple long-term toxicity assay for aquatic contaminants such as DCM using the nematode Panagrellus redivivus was also reported (Samoiloff et al. 1980).

Reproductive and Developmental Toxicity

DCM and its metabolite CO cross the placenta. Maternal blood DCM concentrations were higher than fetal blood DCM concentrations, and maternal and fetal CO concentrations were similar in pregnant rats exposed to 500 ppm DCM. Fetal blood CO concentrations were higher than maternal blood CO concentrations in pregnant rats exposed to 20 ppm CO (Anders and Sunram 1982). A linear relationship between the mean fetal and maternal blood concentrations with exposure level was observed in rats inhaling about 100 to 3,000 ppm DCM for five hours on the 17th day of pregnancy (Withey and Karpinski 1985). CO is known to induce fetal brain damage (Ginsberg and Myers 1974, Sullivan et al. 1993). DCM was reported to induce postnatal behavioral effects in rats administered high doses through inhalation (Bornschein et al. 1980). However, lower doses of inhaled DCM failed to cause embryotoxicity and fetotoxicity in mice, rats, and rabbits (Heppel et al. 1944, Leong et al. 1975, Schardein 1993).

Wistar rats given 0, 0.04, 0.4, or 4 percent DCM in the diet from days zero to 20 of gestation had total intake of 0, 134 ± 13, $1,394 \pm 134$, or $13,626 \pm 1,419$ mg DCM, respectively. No signs of maternal toxicity were seen except reduced body weight gain at the highest dose level and no developmental toxicity was observed (Nishio et al. 1984).

Schwetz et al. (1975) exposed groups of pregnant Sprague-Dawley rats and Swiss Webster mice to 1,250 ppm DCM during days 6 to 15 of gestation for seven hours per day for 10 days total. Maternal effects of increased liver weights were observed in both species. A statistically significant increase in mean maternal body weight (11 to 15 percent) was observed in treated mice. The mice exposed to DCM had no effect on the average number of implantation sites per litter, incidence of fetal resorptions, fetal sex ratios, or fetal body measurements. However, a significant ($p < 0.05$) number of litters had pups with a single extra center of ossification in the sternum (6/12 compared with 3/22 in control litters of mice). Cleft palate and rotated kidneys occurred in 2/12 litters of treated mice only but the incidence was not statistically significant.

Litters of rats exposed to DCM had a significantly decreased incidence of lumbar ribs or spurs (1/19 compared with 8/25 in control litters); and a significantly ($p < 0.05$) increased incidence of delayed ossification of sternum (5/19 compared with 0/25 in controls). Dilated renal pelvis was observed in 5/19 litters of exposed rats compared with 1/25 controls. Both DCM-exposed rats and mice exhibited significant elevation of the COHb level in the maternal and dam blood, but the level returned to control values within 24 hours of cessation of exposure. ATSDR (1993) considered 1,250 ppm as the LOAEL for delayed ossification of sternebrae for both the rat and mouse.

Hardin and Manson (1980) compared the effects of DCM inhalation at 4,500 ppm, six hours per day, seven days per week, for three weeks on the fetuses of Long-Evans rats exposed before mating and during days 1 to 17 of gestation, with those exposed before gestation only. A statistically significant increase in maternal liver weights was observed in rats exposed to DCM during gestation. Litters from rats exposed during gestation only had significantly ($p < 0.05$) lower fetal body weights than controls.

Bornschein et al. (1980) tested some of the offspring of the Long-Evans rats from the Hardin and Manson (1980) study. Behavioral abnormalities have been found in rats exposed antenatally to DCM. The progeny of exposed rats before and/or during gestation exhibited altered rates of habituation to novel environments. These effects suggest that DCM exposure prior to or during pregnancy may influence the processes of orientation, reactivity, and behavioral habituation.

In a two successive generation study, Nitschke et al. (1988b) evaluated reproductive parameters in Fischer 344 rats following inhalation of DCM. Male and female rats of F_0 generation, 30 of each sex, were exposed to 0, 100, 500, or 1,500 ppm DCM for six hours per day, five days per week for 14 weeks, and then mated to produce F_1 generation. From the litters, 30 rats of each sex were exposed to the same concentrations of DCM for 17 weeks, then mated to produce F_2 offspring. Other than decreased fetal body weighs, no adverse effects on subsequent fertility, reproductive parameters, neonatal survival, or neonatal growth were noted in animals exposed to DCM in either the F_0 or F_1 generation. There were no gross treatment-related pathological abnormalities in F_0 and F_1 adults or F_1 and F_2 weanlings, and microscopic examination of tissues from F_1 and F_2 weanlings revealed no lesions attributable to DCM exposure. ATSDR (1993) considered 1,500 ppm as the NOAEL of the study.

The NTP (1986) reported that chronic two-year exposure to a high concentration of DCM for six hours per day, five days per week, was associated with an increased incidence of testicular atrophy in male B6C3F1 mice. Sixty-two percent (31/50) of high-dosed males at 4,000 ppm exhibited this pathology at the time of terminal sacrifice. Testicular atrophy was observed in only eight percent (4/50) of low-dosed male mice at 2,000 ppm, and was not seen in any control animals. In addition, a concentration-related increase in the incidence of ovarian atrophy was detected in female mice. It occurred in 6/50, 28/48, and 32/47 animals of control, low-dose, and high-dose groups, respectively.

In dominant lethal tests, male mice were exposed to DCM at concentrations up to 200 ppm by inhalation for two hours per day, five days per week, for up to six weeks. No microscopic lesions were found in the testes (Raje et al. 1988). Pregnant Fischer 344 rats gavaged with DCM on gestation day 6 to 19 exhibited reduced maternal weight gain through the first 10 days of dosing as well as reduced extrauterine weight gains throughout treatment at 450 mg/kg-day, but no maternal toxicity was observed at 337.5 mg/kg-day except one death immediately after dosing in each of the two DCM-treated groups. DCM did not cause full-litter resorptions (Narotsky and Kavlock 1995). The activity of plasma butyrylcholinesterase, which is inversely related to testosterone level, produced in the liver was significantly increased in male but not in female NMR1 mice after inhalation exposure to 300 ppm DCM for 30 days (Kjellstrand et al. 1985).

The adverse reproductive effects of CO as a metabolite of DCM have not been reported. However, Fechter and Annau (1997) demonstrated that low levels of CO exposure produced damaged fetal CNS growth and reduced fetal growth in animals. CO inhalation in pregnant rabbits increased fetal mortality and decreased live birthweight (Astrup et al. 1972). These effects suggest that the fetus may be especially sensitive to the toxic effects of DCM through its metabolism to CO.

Brown-Woodman et al. (1998) examined embryotoxicity of DCM in an *in vitro* assay. Rat embryos were explanted on day 10.5 of gestation and cultured in rat serum for 40 hours of their critical period of organogenic development. Various concentrations of DCM were added to the culture. Embryonic growth and development were assessed at the end of the culture period, and embryotoxicity level for DCM was estimated to be 6.54 μmole/mL. The author also estimated the equivalent exposed human blood level to be 0.174 μmole/mL.

A study using chicken embryos reported that DCM applied to the air space of the fertilized eggs at two, three, and six days of incubation disrupted embryogenesis with macroscopic malformations in a dose-related manner. The approximate LD_{50} was over 100 μmole per egg (Elovaara et al. 1979). DCM enhanced viral transformation in Syrian hamster embryo cells (Hatch et al. 1983). OSHA (1997) considered this result as suggestive evidence of potential teratogenicity. OSHA (1997) also concluded from the animal studies that maternal exposure to high concentrations of DCM during pregnancy might have some adverse effects on the offspring, in particular with regard to behavioral effects.

According to the proposed Nordic criteria for reproductive toxicity, DCM is considered to be classified as Group 3, an agent nonclassifiable as to its reproductive toxicity since it has been investigated but cannot be classified in Group 1 or 2 (Taskinen 1995). The German Commission for the investigation of health hazards of chemicals in the work area classifies DCM reproductive toxicity as D implying "the risk is not possible to evaluate due to insufficient data even though the available data indicate a trend of toxicities" (Barlow and Sullivan 1982, Sullivan et al. 1993).

Genotoxicity

DCM has been evaluated in forward and reverse mutation assays in bacteria, in tests of recombination and reverse mutation in yeast, and for its activity in the sex-linked recessive lethal mutation assay with fruit flies. There is some evidence that DCM is mutagenic in nematodes and plants. A series of studies with cultured mammalian cells has examined DCM's clastogenic potential, as well as its ability to induce DNA damage and repair (Arthur D. Little, Inc. 1985, ECETOC 1987, IARC 1986, 1987, Green 1980, McCarroll et al. 1983, Strobel and Grummt 1987). Mutational specificity as the mechanistic basis of the hepatic and pulmonary carcinogenicity of DCM in female B6C3F1 mice was investigated and discussed in a series of publications (Devereux et al. 1993, Foley et al. 1993, Hegi et al. 1993, Kari et al. 1993) edited by Anderson and Maronpot (1993).

OSHA (1997) concluded that the evidence that DCM is genotoxic is compelling, and "Evidence for the interaction of DCM or DCM metabolites with DNA (producing mutations or toxicity) is consistent with a genotoxic mechanism for the carcinogenic action of DCM, rather than a non-genotoxic action (i.e., by acting as a promoter, increasing cell turnover)." Detailed reviews of the genotoxicity of DCM are also available in documents from Broome and Sivak (1988), DHS (1989), and U.S. EPA (1985a).

Mutations

Bacteria

In the Ames test of reverse mutation with *Salmonella typhimurium*, vapor phase exposure of strains TA100, TA1535, or TA98 to DCM has consistently resulted in positive evidence of mutagenic activity. Strains TA1537 and TA1538 were negative. These results have been obtained both with and without the presence of exogenous metabolic activation (Barber et al. 1980, Gocke et al. 1981, Green 1981, 1983, Jongen et al. 1978, 1982, Kanada and Uyeta 1978, Kirwin and Thomas 1980, McGregor 1979, Nestmann et al. 1981, Osterman-Golkar et al. 1983, Simmon et al. 1977, Simmon and Kauhanen 1978, Snow et al. 1979).

The mutagenic responses at the highest dose of 115 μmoles per plate for the above indicated strains were 0.0006, 0.006, and 0.003 revertants per μmole, respectively (U.S. EPA 1985a), indicating that DCM is a weak mutagen in *Salmonella*. Direct addition of liquid DCM to incubation media has given negative results with *Salmonella,* presumably related to inadequate exposure due to the volatility of DCM (Barber et al. 1981, McGregor 1979, Nestmann et al. 1980, Rapson et al. 1980). Positive relationship between exposure time and metabolism of DCM in *Salmonella typhimurium* was reported (Jongen 1984).

The metabolic activation of DCM in the *Salmonella* system was mediated by GST enzymes (DeMarini et al. 1997, Green 1983). Direct-acting mutagenicity of DCM was induced in TA100 by bacterial GST enzymes similar to those known in the rat (Osterman-Golkar et al. 1983). Indirect-acting mutagenicity was derived from glutathione conjugation of DCM with increased concentrations of post-mitochondrial supernatant (Green 1983). Graves et al. (1994a) found that there was approximately a two-fold decrease in mutations in glutathione-deficient *Salmonella typhimurium* strain TA100/NG11, compared to TA100. Mutation rates returned to normal when bacteria were supplemented with exogenous glutathione. S-chloromethylglutathionate conjugates, metabolites of the GST pathway, were responsible for mutagenesis in *Salmonella typhimurium* strains (Jongen et al. 1982). Experiments with *Escherichia coli* strains implicated the metabolite formaldehyde as the active mutagen. Colony probe hybridization performed on approximately 100 revertants in strain RSJ100, a derivative of TA1535 cloned with the rat GSTT1-1 gene, induced by DCM demonstrated that 96 to 100 percent of the mutations were GC $\rightarrow$ AT transitions, and 87 to 100 percent were at the second position of the CCC/GGG target. In contrast, only 15 percent of mutants induced by DCM were GC $\rightarrow$ AT transitions in the absence of the GSTT1-1 gene in TA100 (DeMarini et al. 1997).

However, similar experiments in DCM using *Salmonella* strain TA100/NG54 with and without Aroclor induced male Fischer 344 rat liver S9 did not demonstrate such clear results on the mechanism (Dillon et al. 1992). In the absence of S9, DCM mutagenicity was similar in TA100 compared with TA100/GSTA1-1 and TA100/GSTP1-1, the two strains expressing human alpha and pi class GST, respectively (Simula et al. 1993).

Mutagenicity of mixtures of DCM and 3-methylcholanthrene, a polycyclic aromatic hydrocarbon substrate of P450 enzymes, in the Ames test with TA98 and TA100 in the presence of S9 increased by 25 and 18 percent, respectively, relative to mutagenicity of DCM only (Kevekordes et al. 1998). In *Salmonella* TA 1535, expression of rat and bacterial DCM-active GST from a plasmid yielded up to 5.3 times more reversions to histidine prototrophy in the transconjugant expressing the rat enzyme. GST-mediated conversion of DCM to formaldehyde was lower in cell-free extracts of the transconjugant expressing the rat GSTT1 than in the corresponding strain expressing the bacterial DCM dehalogenase. This provided new evidence that formaldehyde was not the main toxicant associated with GST-mediated DCM conversion, and indicated that an

intermediate in the transformation of DCM by GST, presumably S-chloromethylglutathione, was responsible for the observed effects (Gisi et al. 1999).

DCM was mutagenic in Ames test strain TA 1535 that produced rat theta GST5-5 protein (Thier et al. 1993). Oda et al. (1996) developed a new *Salmonella typhimurium* strain NM5004 expressing 52-fold greater GST 5-5 activity than the original umu strain TA1535/pSK1002. Using the SOS/umu test system, DCM induced umuC gene expression more strongly in the NM5004 than in the original *Salmonella* strain, an observation in accord with the earlier results of Thier et al. (1993).

DCM exerted mutagenic and lethal effects in the L-arabinose resistance (Ara) forward mutation test of *Salmonella typhimurium*. The mutagenicity decreased in the presence of an exogenous mammalian S9 mixture suggesting that DCM can be metabolized to a mutagen by enzymes of the bacteria, and the mutagen can be metabolically scavenged by reactions with components from the S9 system (Roldan-Arjona and Pueyo 1993). DCM activated SOS-inducing activity in *Salmonella typhimurium* TA1535/pSK1002 (Nakamura et al. 1987).

In *Escherichia coli*, DCM was mutagenic to strains WU361089 and K49, but not to Sd-4 (Osterman-Golkar et al. 1983). DCM activated by mouse liver homogenate S9 was mutagenic in wild type K12 (AB1157). Mouse liver S9 also enhanced the cell-killing effect of DCM in the uvrA and a recA/uvrA double mutant (AB2480) which is sensitive to DNA damages (Graves et al. 1994a). Liver S9 from Aroclor induced male Fischer 344 rats activated DCM mutagenicity in strain WP2 uvrA pKM101 (Dillon et al. 1992). Forward mutations induced by DCM in the N-terminal region of the lacI gene of *Escherichia coli* were reported using excision repair proficient uvr+ and excision repair defective $uvrB^-$ strains (Zielenska et al. 1993).

No evidence of mutagenicity was found when *Bacillus subtilis* was exposed to DCM with or without the addition of metabolic activation (Kanada and Uyeta 1978).

Lower Eukaryotes

DCM increased the incidence of mitotic recombination, gene conversion and reverse mutation in yeast *Saccharomyces cerevisiae* strain D7 (Callen et al. 1980) but not in *Saccharomyces cerevisiae* D3 (Simmon et al. 1977). This discrepancy may be explained on the basis of strain-specific differences in endogenous levels of cytochrome P450 or differences in exposure conditions. The U.S. EPA (1985a) concluded that DCM is probably mutagenic to yeast.

Fruit flies

Two feeding studies, one injection study, and an inhalation study were conducted with DCM to detect sex-linked recessive lethal mutations in *Drosophila melanogaster* fruit flies. DCM was negative in the inhalation study (Kramers et al. 1991) and the injection study (Abrahamson and Valencia 1980), but was weakly positive for gene mutation in the feeding study by Gocke et al. (1981). DCM induced recombination in three strains of *Drosophila melanogaster*, a wild-type insecticide susceptible laboratory strain (Leiden-S, ST) and two insecticide resistant strains (Hikone-R, HK, and Hagg-R, HG) with high cytochrome P450 dependent bioactivation capacities (Rodriguez-Arnaiz 1998).

Mammalian Systems

DCM did not induce mutations in mammalian systems of cultured human peripheral lymphocytes, Chinese hamster ovary (CHO) V79 cells, and mouse lymphoma L5178Y cells both with and without the presence of exogenous metabolic activation (Jongen et al. 1981).

Chromosome Effects

DCM induced elevated micronucleus frequencies in the cytochalasin B-blocked micronucleus assay using two genetically engineered cell lines deriving from parental cell line AHH-1 (Doherty et al. 1996). The MCL-5 cell line expresses cDNAs encoding human CYP1A2, 2A6, 3A4, 2E1 and microsomal epoxide hydrolase, and the h2E1 cell line contains a cDNA for CYP2E1. In addition, DCM metabolites can be produced in these two cell lines. The methodology demonstrated the ability of metabolically competent cell lines expressing cDNAs encoding cytochrome P450 isoenzymes to metabolize DCM to genotoxic species.

DCM given by gavage (1,250, 2,500, or 4,000 mg/kg) to male and female mice revealed no significant increase in the incidence of micronuclei in polychromatic erythrocytes in bone marrow of animals from any dose group (Sheldon et al. 1987). However, an earlier study reported that DCM induced micronucleated polychromatic erythrocytes in mice (Gocke et al. 1981).

Inhalation exposure of B6C3F1 mice to DCM for 10 days at concentrations of 4,000 ppm or 8,000 ppm resulted in significant increases in frequencies of sister chromatid exchange (SCE) in lung cells and peripheral blood lymphocytes, chromosomal aberrations in lung and bone marrow cells, and micronuclei in peripheral blood erythrocytes (Allen et al. 1990). In the same study after three months of inhalation exposure to 2,000 ppm DCM, increased SCEs in lung cells and micronuclei in erythrocytes were observed in the mice.

A single subcutaneous injection of DCM at 2,500 or 5,000 mg/kg did not induce SCEs in bone marrow cells (Allen et al. 1990). Following intraperitoneal injections of DCM at 100, 500, 1,000, 1,500, and 2,000 mg/kg in C57B1/6J mice, no increase of the frequencies of SCEs or chromosomal aberrations were observed (Westbrook-Collins et al. 1990).

Thilagar and Kumaroo (1983) found DCM inhibited mitosis and induced chromosomal aberrations, but had no significant effect on the frequency of SCE in CHO cells. Other studies reported increases in the frequencies of SCE both with and without metabolic activation in cultured human peripheral lymphocytes, CHO V79 cells, and mouse lymphoma L5178Y cells (Jongen et al. 1981, Thilagar et al. 1984a).

The GSTT-1 enzyme polymorphism of glutathione conjugation influenced the induction of SCE in human lymphocytes *in vitro* by DCM. Higher frequencies of SCE were seen in blood samples obtained from nonconjugators compared with conjugators, and having the ability to conjugate DCM resulted in protection for the cytogenic effects (Hallier et al. 1993).

DCM was found to induce a dose-related increase in the number of chromosomal aberrations per cell, and in the percentage of cells with aberrations *in vitro*. It induced extensive chromosomal aberrations, with and without metabolic activation, in cultured human peripheral lymphocytes, CHO cells, and mouse lymphoma L5178Y cells (Thilagar et al. 1984b). However, Gocke et al. (1981) did not observe chromosomal aberrations in bone marrow cells in mice exposed to DCM *in vivo*.

Mouse liver cytosol, in reactions requiring glutathione, mediated metabolism of DCM to produce DNA single strand breaks and cause mutations at the HPRT locus of CHO cells. The authors concluded that the DNA damages and mutations were induced by the glutathione conjugate of DCM (Graves and Green 1996). The same group (Graves et al. 1996) also compared the mutation spectrum of DCM, formaldehyde and 1,2-dibromoethane to investigate the involvement of the glutathione conjugates in DCM mutagenicity. The results suggested that the S-chloromethylglutathionate metabolites play a major role in DCM mutagenesis.

As part of a lifetime inhalation bioassay of DCM, Burek et al. (1984) evaluated the chromosomal effects on Sprague-Dawley rats exposed to 500, 1,500, or 3,500 ppm DCM, six hours per day, five days per week for six months. Bone marrow cells were examined from five males and five females from each exposure group. Inhalation of DCM did not significantly increase the frequency of the total number of cells with chromosomal aberrations or the number of cells with a specific type of aberration.

DNA Binding, Damage and Repair

DCM induced DNA damage detectable as single strand breaks by alkaline elution in freshly isolated hepatocytes from mice and rats. The single strand breaks were also detectable in hepatocyte DNA from mice which inhaled 4,000 ppm DCM for six hours but not in hepatocytes in rats similarly exposed (Graves et al. 1994b). GST-mediated metabolism of DCM by mouse liver cytosol S100 caused DNA single strand breaks in CHO cells. Interspecies differences in DCM-induced DNA damage, assessed *in vivo* and *in vitro*, indicate that the induction of single strand DNA breaks by DCM correlated with the metabolism of DCM by the GST pathway (Graves et al. 1995).

Covalent binding of metabolites of DCM to subcellular macromolecules in hepatocytes was reported (Cunningham et al. 1981). Green et al. (1988) measured the binding of radioactivity to lung and liver DNA isolated from rats and mice exposed to 4,000 ppm ^{14}C-DCM by inhalation for three hours. Analysis of lung and liver tissue at 6, 12, or 24 hours after the start of exposure detected radioactivity in DNA in all samples from both species. There was no evidence of DNA alkylation in liver and lung cells in rats and mice exposed to DCM *in vivo*. All of the radiolabel detected in samples was due to incorporation of ^{14}C into DNA via normal pathways of biosynthesis (Green et al. 1988, Ottenwalder and Peter 1989).

Jongen et al. (1981) did not observe unscheduled DNA synthesis (UDS) in human primary fibroblasts or in CHO V79 cells without exogenous activation. UDS was not observed in livers of mice and rats exposed to DCM (Trueman and Ashby 1987). UDS was negative in human peripheral lymphocytes with and without activation (Perocco and Prodi 1981). Exposure to DCM did not affect either scheduled or unscheduled DNA synthesis in cultured human lymphocytes (Perocco and Prodi 1981), and did not damage mouse or rat hepatocyte DNA in vivo or *in vitro* (Andrae and Wolff 1983, Trueman and Ashby 1987). However, Kitchin and Brown (1989) observed hepatic DNA damage in female rats dosed with DCM by gavage at 1,275 mg/kg.

Other Genotoxic Effects

Negative results were observed in dominant lethal tests in mice. Male mice were exposed to DCM at concentrations up to 200 ppm by inhalation for two hours per day, five days per week, for up to six weeks, or were treated by repeated subcutaneous injections. Fertility effect, dominant lethality, or chromosomal damage in testes was not demonstrated (Raje et al. 1988).

Devereux et al. (1993) identified activated ras proto-oncogenes in both DCM induced liver and lung tumors in female B6C3F1 mice exposed by continuous inhalation of 2,000 ppm for six hours per day, five days per week for 104 weeks. Some lung tumors in DCM-exposed mice exhibited point mutations in activated K-ras oncogenes. In contrast, liver tumors induced by DCM contained mutations in H-ras genes that were typical of what were observed in spontaneous liver tumors. However, the low number of spontaneous tumors available in this study limited the interpretation of the data. An association between K-ras gene activation and allelic imbalances on chromosome 6 was observed for B6C3F1 lung tumors (Hegi et al. 1994).

Mutations in ras genes in experimental tumors of rodents were evaluated for *in vivo* mutagenesis and

carcinogenesis. DCM-induced liver neoplasms had an H-ras mutation profile at codon 61 similar to that of spontaneous tumors, suggesting that DCM promoted cells with "spontaneously initiated" ras mutations (Sills et al. 1999).

Loss of heterozygosity (LOH) at markers near the p53 gene on chromosome 11 was detected in seven out of 54 DCM-induced lung carcinomas from female B6C3F1 mice. Allele loss was observed at all six markers, thus, these chromosomal alterations were likely to have resulted from mitotic nondisjunction (Hegi et al. 1993). Alleotype analysis of mouse lung carcinomas revealed frequent allelic losses on chromosome 4 (Hegi et al. 1994). Davis et al. (1994) examined 142 spontaneous and chemically-induced liver tumors isolated from the B6C3F1 mouse for LOH at 78 polymorphic loci and compared these results to genetic changes known to occur in human hepatocellular carcinoma. Approximately a third of the 142 mouse tumors exhibited LOH suggesting that tumor suppressor gene inactivation might be involved in the formation of mouse liver tumors. Losses observed on chromosomes 5 and 8, syntenic to human chromosomes 4 and 16, suggest tumor suppressor genes that are common to liver carcinomas from both species.

Absence of covalent binding to mouse liver DNA *in vivo* was reported. Male B6C3F1 mice, gavaged with DCM in corn oil at 1,000 mg/kg or inhaling 4,000 ppm for two hours, did not show S-phase DNA synthesis induction (Lefevre and Ashby 1986, 1989). Replicative DNA synthesis and reduced cell turnover in lung cells were reported in female B6C3F1 mice exposed to 2,000 ppm DCM by inhalation for two to 26 weeks to induce pulmonary carcinogenesis, but no treatment-induced cell proliferation in the latent period was observed (Kanno et al. 1993). Inhalation exposure to a hepatocarcinogenic concentration of DCM did not induce sustained replicative DNA synthesis in hepatocytes of female mice indicating that the enhanced cell proliferation is not a major mechanism associated with DCM hepatocarcinogenicity (Foley et al. 1993).

B6C3F1 mouse lung has a higher proportion of Clara cells than the human lung and these cells have been implicated as a main metabolic site for inhaled DCM in mouse lungs (Green 1990a, 1991). Exposure to DCM resulted in cytotoxicity, cell division, and metabolic changes in the abundant Clara cells that are not seen in other lung cells (Foster et al. 1994). DNA synthesis increased in bronchiolar cells of mice that inhaled DCM but no significant increases occurred in the alveolar cells (Foster et al. 1992). However, DNA damage detected in lungs of mice exposed to 2,000 ppm was not observed in Clara cells isolated from mice exposed to 4,000 ppm DCM (Devereux et al. 1989). OSHA (1997) raised doubts that the evidence could discount the carcinogenicity of DCM in mice demonstrated by the NTP (1986).

DCM increased neoplastic transformation frequencies in the BALBc/-3T3 Transformation Assay (Sivak 1978). DCM at 175 μM increased the frequency of chromosomal aberrations in the onion bulb root cells using the Allium anaphase-telophase test. DCM was one of the commonly found components in industrial wastewater and the assay was used for screening genotoxicity of complex environmental mixtures (Rank and Nielsen 1994).

Carcinogenicity

The goal of this section is to review the findings of the seven available animal cancer bioassays that have been completed on DCM. Some of the bioassays are discussed in more detail because they are used in the quantitative risk assessment to derive a health-based drinking water level for DCM. The carcinogenicity of DCM to animals has also been reviewed by the U.S. EPA (1985a, 1985b), NTP (1986), ACGIH (1986, 1990, 1995) with NIOSH (1992) and OSHA (1997), FDA (1989), CPSC (1986, 1987, 1990), IARC (1986, 1987, 1999), and the DHS (1989). The U.S. EPA considered the animal carcinogenicity data on DCM sufficient for classifying DCM as a Group B2 probable human carcinogen. IARC (1999) concluded, "…bioassays …by inhalation

exposure in both mice and rats provided sufficient evidence for carcinogenicity to experimental animals."

Serota et al. (1986a, 1986b)

This is a series of two-year laboratory animal studies performed by Hazleton Laboratories America, Inc., sponsored and reported to the U.S. EPA by the National Coffee Association (1982, 1983) and later published by Serota et al. (1986a, 1986b). A summary of the data is presented in Table 4 for rats and Table 5 for mice.

Rats

Serota et al. (1986a) administered DCM in the drinking water of Fischer 344 rats at target levels of 5, 50, 125 and 250 mg/kg-day (Groups Three to Six). A total of 500 rats, including 85 rats of each sex per group, were exposed continuously for 104 weeks. Two control groups, Group One with 85 rats and Group Two with 50 rats, consisted of a total of 135 rats per sex at a target level of zero DCM mg/kg-day. An additional group (Group Seven) of 25 rats of each sex received DCM at 250 mg/kg-day for 78 weeks followed by a 26-week recovery period. The actual mean daily consumption levels of DCM in the drinking water were estimated in Groups Three to Six as 6, 52, 125, and 235 mg/kg-day for males, and 6, 58, 136, and 263 mg/kg-day for females. The estimated level for the recovery Group Seven was 232 and 269 mg/kg-day for males and females, respectively.

The overall survival rates among males and females were 76 and 71 percent, respectively. Throughout the study, small but significant decreases in body weight, body weight gain, and water consumption were reported for both male and female rats in the two highest dose groups. No treatment-related findings were reported with respect to survival, clinical observations, ophthalmologic findings, gross necropsy results, or organ weight data. Histomorphological alterations of the liver were observed, including an increased incidence of areas of cellular alteration in Groups Four, Five, Six and Seven, and an increased incidence of fatty change in Groups Five and Six.

In female rats treated with DCM at 50 or 250 mg/kg-day the incidence of combined hepatocellular carcinoma and neoplastic nodules was statistically significantly increased (0/134, 1/85, 4/83, 1/85, and 6/85 in the five dose groups of 0, 5, 50, 125, and 250 mg/kg-day, respectively) when compared with matched control Groups One and Two ($p < 0.05$ and $p < 0.01$, respectively). There were no significant treatment-related increase in the incidence of hepatocellular carcinomas alone in treated females (0/134, 0/85, 2/83, 0/85, 2/85). Compared to control animals, there was no increase in the incidence of liver tumors in treated male rats.

The incidences of hepatocellular carcinoma and neoplastic nodules in concurrent historical controls based on 324 untreated female Fischer 344 rats were 1.7 percent (4/324) and 6.3 percent (21/324), respectively. The combined incidence of hepatocellular carcinoma and neoplastic nodules in controls and the four dose groups (472 rats: four with carcinoma and eight with neoplastic nodules) was similar to that for historical controls (419 rats: five with carcinoma, 19 with neoplastic nodules). No liver tumors were observed in the controls. The authors did not consider the increase in liver tumors in female rats to be biologically significant but the U.S. EPA (1985a) concluded that the results represent a borderline carcinogenic response to DCM by Fischer 344 female rats.

Mice

In the same National Coffee Association study (Serota et al. 1986b), a total of 1,000 B6C3F1 mice received DCM in drinking water at target levels of 60, 125, 185, and 250 mg/kg-day for 104 weeks. Treatment groups, Group Three to Six, consisted of 100, 50, 50, and 50 female mice and 200, 100, 100, and 125 male mice each for low to high dose. One hundred females, 50 each in Group One and Two, and 125 males, 60 in Group One and 65 in Group Two, served as controls at the target level of zero. The mice in each treatment group consumed concentrations of DCM within acceptable ranges of the target levels throughout the course of the study. The actual mean daily consumption levels of DCM in the drinking water were estimated in Groups Three to Six as 61, 124, 177, and 234 mg/kg-day for males, and 59, 118, 172, and 238 mg/kg-day for females.

Overall survival rates among males and females were 82 percent and 78 percent, respectively. No significant differences in body weight were observed over the course of the study in DCM-treated male or female mice compared to controls and no treatment-related clinical effects were observed during the study. The incidence of mice exhibiting convulsions ranged from 74.1 percent in the 125 mg/kg-day dosed males to 100 percent in the Group One female controls. A statistically significant increase in mean leucocyte count was seen in both males and females dosed at 250 mg/kg-day for 52 weeks. A nonsignificant increase in the number of male mice with Harderian gland neoplasms was observed in groups exposed to 125 mg/kg-day and 250 mg/kg-day.

The liver was the target organ and treatment related histomorphological alterations were observed in both sexes dosed with DCM at 250 mg/kg-day. Similarly, as reported previously in a 90-day drinking water study (Kirschman et al. 1986), an increase in the liver fat content was observed. Females treated with DCM did not show an increased incidence of proliferative hepatocellular lesions.

Male mice had a statistically significant ($p = 0.0114$) increased incidence of combined neoplastic nodules and hepatocellular carcinoma (24/125, 51/200, 30/100, 31/99, 35/125) compared to the first control group but it was not significant when compared to a second control group or to the combined incidence from both control groups. The increase was not dose-related, but the pairwise comparisons for the two mid-dose groups were reported to be statistically significant (U.S. EPA 1985a). A statistically significant increase in the incidence of hepatocellular adenomas and carcinomas was observed in groups treated with 125 mg/kg-day ($p = 0.042$) and 185 mg/kg-day ($p = 0.027$), and a borderline significant increase was seen in the 250 mg/kg-day dose group. The hepatocellular carcinoma incidence alone for male mice (which was about 55 to 65 percent of the total) was not significantly elevated. Female mice did not have increased liver tumor incidence.

According to the authors, DCM did not induce a treatment-related carcinogenic response in B6C3F1 mice under the conditions of this study. However, the U.S. EPA (1985a) determined that the results of this study are indicative of a borderline carcinogenic response to DCM by B6C3F1 male mice.

Table 4. Carcinogenicity Bioassay of Dichloromethane Based on Drinking Water Ingestion in Female Fischer 344 Rats (Serota et al. 1986a)

Concentration [a, b] (mg/kg-day)	Tumor type	Incidence	Significance [c]
0	Liver adenoma	0/85	
0		0/49	
5 [a] (6) [b]		1/85	
50 (58)		2/83	
125 (136)		1/85	
250 (263)		4/85	
250/Recovery [d] (269)		2/25	
Historical control		19/419	
0	Liver carcinoma	0/85	
0		0/49	
5 (6)		0/85	
50 (58)		2/83	
125 (136)		0/85	
250 (263)		2/85	
250/Recovery [d] (269)		0/25	
Historical control		5/419	
0	Liver adenoma and carcinoma combined	0/85	
0		0/49	
5 (6)		1/85	
50 (58)		4/83	$p < 0.05$
125 (136)		1/85	
250 (263)		6/85	$p < 0.01$
250/Recovery [d] (269)		2/25	
Historical control		24/419	

[a] DCM given in deionized drinking water in water bottles for 104 weeks, target level.
[b] Expressed in brackets, estimates of mean daily consumption determined from measured DCM concentrations in drinking water, the actual body weights and water consumption values.
[c] Fisher Exact Test.
[d] Exposed to DCM for 78 weeks and allowed to recover.

Table 5. Carcinogenicity Bioassay of Dichloromethane Based on Drinking Water Ingestion in Male B6C3F1 Mice (Serota et al. 1986b)

Concentration [a, b] (mg/kg-day)	Tumor type	Incidence	Significance[c]
0	Multi focal hyperplasia	4/60	
0		6/65	
60[a] (61)[b]		14/200	
125 (124)		4/100	
185 (177)		10/99	
250 (234)		13/125	
0	Liver adenoma	6/60	
0		4/65	
60 (61)		20/200	
125 (124)		14/100	
185 (177)		14/99	
250 (234)		15/125	
0	Liver carcinoma	5/60	
0		9/65	
60 (61)		33/200	
125 (124)		18/100	
185 (177)		17/99	
250 (234)		23/125	p = 0.0114 [d]
Historical control		16.1% (5-34%)[e]	
0	Liver adenoma and carcinoma combined	11/60	
0		13/65	
60 (61)		51/200	
125 (124)		30/100	p = 0.042
185 (177)		31/99	p = 0.027
250 (234)		35/125	p = 0.031
Historical control		17.8% (5-40%)[e]	

[a]DCM given in deionized drinking water in water bottles for 104 weeks, target level.
[b]Expressed in brackets, estimates of mean daily consumption determined from measured DCM concentrations in drinking water, the actual body weights and water consumption values.
[c] Fisher Exact Test using combined control incidence of 24/125.
[d]Fisher Exact Test, p = 0.0114 compared to the first control Group One in Serota et al. (1986b).
[e]Average based on a total of 354 untreated male mice, ranges expressed in brackets.

Maltoni et al. (1986, 1988)

Between 1979 and 1983 Maltoni et al. (1986, 1988) conducted three carcinogenicity experiments on mice and rats. DCM of 99.973 percent purity was administered orally by stomach tube and by inhalation, and animals were observed until natural death of the animal.

In experiment BT3002, 50 Sprague-Dawley rats per sex per group were exposed by gavage to DCM in olive oil at 100 and 500 mg/kg-day, four to five days per week, for 64 weeks. Excess mortality was observed in male ($p < 0.01$) and female (not significant) rats administered DCM at 500 mg/kg, and for this reason treatment was stopped after 64 weeks. No effect of DCM on body weight was observed in either sex at any dose level. DCM did not affect the percentage of rats with benign or malignant tumors. A higher frequency of malignant mammary tumors, primarily adenocarcinomas, was observed in treated females at 500 mg/kg. This increase was not statistically significant.

In experiment BT3003, Swiss mice, 50 animals per sex per group, were exposed by gavage to DCM in olive oil, at 100 and 500 mg/kg-day, four to five days per week, for 64 weeks. The experiment ended after 82 weeks. Excess mortality was observed in male and female mice ($p < 0.01$) exposed to DCM at both dose levels. As with the rats, treatment was terminated after 64 weeks. A decrease in body weight was observed in both male and female mice. DCM did not increase the number of animals with benign and malignant tumors. DCM did not increase the incidence of mammary carcinomas, leukemia, or hepatomas, but a dose-related increase in pulmonary adenomas was observed in male mice. When mortality was taken into account, this increase in lung adenomas became significant ($p < 0.05$) for male mice treated at the higher dose level that died during weeks 52 to 78 of the experiment.

In experiments BT4005-4006, Sprague-Dawley rats were exposed to DCM by inhalation. A group of 54 rats, including 21 pregnant females, were exposed to 100 ppm for four hours per day, five days per week for seven weeks, and then seven hours per day, five days per week, for 97 weeks, which was 104 weeks in total. The breeders were 13-week old at the start of the experiment. Embryos in pregnant females were exposed transplacentally during pregnancy, and the offspring were exposed concurrently during weaning by inhalation. No excess mortality was found in the exposed groups, and no effect on body weight was observed. No significant exposure-related differences in the incidence of benign or malignant tumors were noted among breeders and offspring exposed to DCM for 104 weeks. A non-significant increase in the total number of malignant mammary tumors was observed in rats treated for 104 weeks.

National Toxicology Program (1986)

The NTP (1986) two-year inhalation studies of DCM in rodents have been summarized by Mennear et al. (1988). A summary of the data is presented in Table 6 for rats and Table 7 for mice. NTP (1986) has conducted a carcinogenicity study of DCM of 99 percent purity. An earlier gavage bioassay of DCM conducted by NTP (1982) is summarized in the NTP (1986) report but has not been published because of high mortality, much of which was attributed to gavage accidents.

Rats

Groups of 50 each male and female Fischer 344/N rats were exposed to DCM by inhalation, six hours per day, five days per week for two years. Exposure concentrations were 0, 1,000, 2,000,

and 4,000 ppm. All surviving rats were sacrificed during week 111 to 112. Survival of male rats in all groups was low (18 percent to 34 percent), however, this apparently was not treatment-related. Survival was decreased in a treatment-related fashion for female rats. The survival of the high dose group of female rats was significantly lower ($p < 0.01$) than that of the controls after week 100. This reduced survival in rats may have been due to the relatively high incidence of leukemia reported for all dose groups. Mean body weights of dosed and control rats of each sex were comparable throughout the study.

Mammary adenomas and fibroadenomas were significantly increased in male and female rats after survival adjustment, as were mononuclear cell leukemias in female rats. Mammary gland fibroadenomas, and adenomas and fibroadenomas combined in male and female rats occurred with a significant positive trend ($p < 0.001$, Life Table test), and the incidences in high dose males and females were significantly greater than those in controls.

Mammary gland adenomas or fibroadenomas were first observed at week 93 in males and week 65 in females. Combined subcutaneous fibromas, and fibromas or sarcomas located in the mammary region in male rats also occurred with a significant positive trend ($p < 0.001$), although the incidences in dosed groups were not significantly greater than in controls. These fibromas or sarcomas were first observed 89 weeks after the start of the experiment. When combined, subcutaneous tumors and other mammary gland tumors occurred in male rats with a significant positive trend ($p < 0.001$) and the incidence in the high dose group was significantly greater than in the controls.

Mesotheliomas of the tunica vaginalis occurred in male rats with a significant positive trend ($p = 0.09$, Life Table test), and the incidence of these tumors in the high dose group was significantly greater than in controls. These tumors were first observed at week 69. The incidence of mesothelioma arising from all sites increased significantly ($p = 0.02$), and the incidence in the mid- and high-dose groups was significantly higher ($p = 0.038$ and $p = 0.031$, respectively) than in the controls. However, the incidence of these tumors was not statistically significant when compared to historical controls.

Mice

Groups of 50 each of male and female B6C3F1 mice were exposed to DCM by inhalation, six hours per day, five days per week for two years. Exposure concentrations were 0, 2,000, and 4,000 ppm. All surviving mice were sacrificed during week 112 to 113. Survival was decreased in a treatment-related fashion for male and female mice. For mice, the mean initial body weight of males in the high dose group was 15 percent lower than that of controls. Final mean body weights of high dose male mice and dosed female mice were 10 percent to 17 percent lower than those of concurrent controls. The survival of male and female mice from the high dose groups was significantly lower ($p < 0.001$, Life Table test) than controls.

Among treated mice of both sexes, there were statistically significant, by Life Table tests, dose-related increases in the incidences of alveolar-bronchiolar adenomas ($p < 0.001$) and carcinomas ($p < 0.001$). Lung adenomas first appeared at 70 weeks in males and 68 weeks in females while carcinomas appeared at 72 weeks in males and 68 weeks in females. No lung tumors were found in the controls, while 70 percent of the high-dose males and 71 percent of high-dose females had multiple tumors. In addition, there were significant dose-related increases in the number of lung tumors per animal (multiplicity) in both sexes of mice.

Male mice had an elevated incidence of hepatocellular adenomas ($p = 0.001$, Life Table test) and carcinomas ($p < 0.001$) of the liver at the high dose, while females had dose-related increases of

both hepatocellular adenomas ($p < 0.001$) and carcinomas ($p < 0.001$). Adenomas and carcinomas were significantly increased alone as well as in combination. Hepatocellular adenomas first appeared in males at 71 weeks and females at 68 weeks; the carcinomas first appeared at 61 weeks in males and 68 weeks in females.

On the basis of significant dose-related increases in the incidences of various benign tumors, e.g., mammary fibroadenomas and adenomas, in DCM-treated rats, the NTP (1986) concluded that there was "some evidence" of carcinogenicity in female rats. In mice, statistically significant increases in the incidences of benign and malignant tumors of the liver and lung provided "clear evidence" of carcinogenicity in both males and females.

Table 6. Carcinogenicity Bioassay of Dichloromethane Based on Inhalation in Rats (NTP 1986)

Strain	Sex (weight)[a]	Concentration[b] (ppm)	Tumor type	Incidence	Significance[c]
Fischer 344	Female (337)	0	Mammary gland fibroadenoma	5/50	
		1,000		11/50	P = 0.028[d]
		2,000		13/50	P = 0.033
		4,000		22/50	P < 0.001
Fischer 344	Female (337)	0	Mammary gland fibroadenoma and adenoma combined	5/50	
		1,000		11/50	P = 0.023[d]
		2,000		13/50	P = 0.0369
		4,000		23/50	P < 0.001
Fischer 344	Male (462)	0	Mammary gland fibroadenoma	0/50	
		1,000		0/50	
		2,000		2/50	
		4,000		4/50	P = 0.02[d]
Fischer 344	Male (462)	0	Mammary gland fibroadenoma and adenoma combined	0/50	
		1,000		0/50	
		2,000		2/50	
		4,000		5/50	P = 0.028

Table 6 (Continued). Carcinogenicity Bioassay of Dichloromethane Based on Inhalation in Rats (NTP 1986)

Strain	Sex (weight)[a]	Concentration[b] (ppm)	Tumor type	Incidence	Significance[c]
Fischer 344	Male (462)	0	Mammary region subcutaneous fibroma	1/50	P = 0.024[e]
		1,000		1/50	
		2,000		2/50	
		4,000		4/50	
Fischer 344	Male (462)	0	Mammary area subcutaneous fibroma and sarcoma combined	1/50	P = 0.008[e]
		1,000		1/50	
		2,000		2/50	
		4,000		5/50	P = 0.050[d]
Fischer 344	Male (462)	0	Mammary gland or subcutaneous tissue: adenoma, fibroadenoma or fibroma	1/50	
		1,000		1/50	
		2,000		4/50	
		4,000		9/50	P = 0.008
Fischer 344	Male (462)	0	Mesotheliomas (all types) of tunica vaginalis	0/50	
		1,000		0/50	
		2,000		4/50	
		4,000		4/50	P = 0.031[e]
Fischer 344	Male (462)	0	Mesotheliomas (all types) all sites	0/50	
		1,000		2/50	
		2,000		5/50	P = 0.028[d, f]
		4,000		4/50	P = 0.031[e, f]

[a]Average terminal (99-week) weight of animals in grams (all dose groups).
[b]Six hours per day, five days per week, for 102 weeks.
[c]Fisher Exact Test, unless otherwise noted.
[d]"Life Table" or "Incidental Tumor" tests reference in NTP (1986).
[e]Based on Trend tests as referenced in NTP (1986).
[f]Not significant when compared to historical controls.

Table 7. Carcinogenicity Bioassay of Dichloromethane Based on Inhalation in Mice (NTP 1986)

Strain	Sex (weight)[a]	Concentration (ppm)[b]	Tumor type	Incidence	Significance[c]
B6C3F1	Male (35.3)	0	Lung alveolar/ bronchial adenoma	3/50	
		2,000		19/50	$p < 0.001$
		4,000		24/50	$p < 0.001$
B6C3F1	Male (35.3)	0	Alveolar/bronchial carcinoma	2/50	
		2,000		10/50	$p = 0.014$
		4,000		28/50	$p < 0.001$
B6C3F1	Male (35.3)	0	Alveolar/bronchial adenoma and carcinoma combined	5/50	
		2,000		27/50	$p < 0.001$
		4,000		40/50	$p < 0.001$
B6C3F1	Male (35.3)	0	Hepatocellular adenoma	10/50	
		2,000		14/49	$p = 0.014$[d]
		4,000		14/49	$p = 0.001$[d]
B6C3F1	Male (35.3)	0	Hepatocellular carcinoma	13/50	
		2,000		15/49	
		4,000		26/49	$p < 0.005$
B6C3F1	Male (35.3)	0	Hepatocellular adenoma and carcinoma combined	22/50	
		2,000		24/49	$p = 0.048$[d]
		4,000		33/49	$p = 0.016$
B6C3F1	Female (33.8)	0	Hepatocellular adenoma	2/50	
		2,000		6/48	
		4,000		22/48	$p < 0.001$
B6C3F1	Female (33.8)	0	Hepatocellular carcinoma	1/50	
		2,000		11/48	$p = 0.001$
		4,000		32/48	$p < 0.001$

Table 7 (Continued). Carcinogenicity Bioassay of Dichloromethane Based on Inhalation in Mice (NTP 1986)

Strain	Sex (weight)[a]	Concentration (ppm)[b]	Tumor type	Incidence	Significance[c]
B6C3F1	Female (33.8)	0	Hepatocellular adenoma and carcinoma combined	3/50	
		2,000		16/48	$p < 0.001$
		4,000		40/48	$p < 0.001$
B6C3F1	Female (33.8)	0	Alveolar/bronchial adenoma	2/50	
		2,000		23/48	$p < 0.001$
		4,000		28/48	$p < 0.001$
B6C3F1	Female (33.8)	0	Alveolar/bronchial carcinoma	1/50	
		2,000		13/48	$p < 0.001$
		4,000		29/48	$p < 0.001$
B6C3F1	Female (33.8)	0	Alveolar/bronchial adenoma and carcinoma combined	3/50	
		2,000		30/48	$p < 0.001$
		4,000		41/48	$p < 0.001$

[a]Average terminal (99-week) weight of animals grams (in all dose groups).
[b]Six hours per day, five days per week, for 102 weeks.
[c]Fisher Exact Test, unless otherwise noted.
[d]Using "Life Table" test as referenced in NTP (1986).

Theiss et al. (1977)

Theiss et al. (1977) investigated the production of pulmonary tumors in Strain A mice by 16 organic contaminants in U.S. drinking water including DCM. Groups of 20 male mice were injected intraperitoneally three times a week with DCM at 0, 160, 400, or 800 mg/kg for a total of 16 or 17 injections over five to six weeks. The animals remaining 24 weeks after the first treatment were sacrificed and examined for lung tumors.

Survival was low in the mid-dose (5/20) and high-dose (12/20) groups. Pulmonary adenomas were found at all three dose levels, but due to the small number of animals surviving, the increase in tumors did not reach statistical significance at the two highest dose levels. At the lowest dose, a significant increase ($p = 0.013$) in the number of tumors was observed.

Burek et al. (1980, 1984)

Rats

Burek et al. (1980, 1984) exposed male and female Sprague-Dawley rats, 129 per sex per dose, to DCM by inhalation at concentrations of 0, 500, 1,500, and 3,500 ppm, six hours per day, five days per week, for two years. Animals were necropsied after 24 months of exposure.

Female rats in the high-dose group had statistically significant ($p < 0.05$, Fisher Exact Test) increased mortality from the 18th through the 24th month compared to controls, which was probably caused by the numerous benign mammary tumors in this group. No other significant differences in mortality were noted, and there were no exposure-related differences in body weights of treated male or female rats.

Female rats at all levels of DCM exposure showed a dose-related increase in the average number of benign mammary tumors per rat (1.7, 2.3, 2.6, 3.0), although the numbers of female rats with benign mammary tumors were not significantly increased in any exposure group. To a lesser degree, benign mammary tumors were also present in males in the 1,500 and 3,500 ppm-exposure groups but the increase was not significant when compared to the concurrent control group. There was no indication of an increased incidence of malignant mammary tumors in either male or female rats exposed to DCM. In evaluating the biological significance of these data, it should be noted that the strain of rats used in this study has a high historical incidence of benign mammary tumors, which normally exceeds 80 percent in females and about 10 percent in males by the end of a two-year study period.

In addition to the benign tumors noted above, male rats from the two highest exposure groups developed a statistically significant positive trend in the incidence of sarcomas of the salivary gland region (1/93, 0/94, 5/91, 11/88). One tumor of this type was found in the controls (1/92) compared to 0/95 in the 500 ppm exposure-group, 5/95 in the 1,500 ppm-group, and 11/97 in males from the 3,500 ppm-exposure group ($p < 0.05$). There is a question as to whether these doses reached the maximum tolerated dose (MTD), particularly in the male rats. This tumor type was first observed at about 60 weeks. A low historical spontaneous incidence (zero to two percent) of this tumor type has been observed in the laboratory of Dow Chemical Company where this study was performed (U.S. EPA 1985a). The 11.3 percent incidence found in the high dose males was higher than both the corresponding controls in this study, and it was also higher than expected based on historical control data for male rats of this strain.

Cancers in the upper respiratory tract including nasal turbinates and larynx were not observed with treatment-related changes (Burek 1984, Reznik 1984). Although the authors (Burek 1984, Burek et al. 1984) suggested that the salivary gland region tumor response may be controversial and related to a common viral infection of the salivary gland causing sialodacryoadenitis, which was present in both control and DCM-exposed rats, U.S. EPA (1985a), in its report of the results of this bioassay, concluded that there was an apparent association between the increased incidence of sarcomas in the salivary gland region of male rats and prolonged exposure via inhalation to 1,500 and 3,500 ppm DCM.

Hamsters

Burek et al. (1980, 1984) also exposed 866 male and female Golden Syrian hamsters, 107 to 109 Syrian hamsters per sex per dose, by inhalation to 0, 500, 1,500, and 3,500 ppm DCM for six hours per day, five days per week, for two years. No exposure-related differences were observed in male hamsters in the number of benign or malignant tumors. A statistically significant increase in the total of number of benign tumors was reported in females exposed to 3,500 ppm; the significance level was not reported. The incidence of lymphosarcoma in female hamsters was not statistically significant in any of the exposed groups (Burek et al. 1984, U.S. EPA 1985a).

There is a question as to whether these doses reached the MTD, particularly in the hamsters. Among the criticisms of this study made by the U.S. EPA (1985a) was that there is no evidence that a MTD was used. Furthermore, relatively few mammary gland tissues samples were examined in the control and exposure groups. No tumors were identified in these tissues, but

because of the small number of samples, it is not possible to know whether this is a valid interpretation of the effect of DCM on hamster mammary gland tissue.

Nitschke et al. (1982, 1988a)

In another study by Dow Chemical Company first reported to the U.S. EPA in 1982 (Nitschke et al. 1982, 1988a), 90 male and 108 female Sprague-Dawley rats were exposed by inhalation to 0, 50, 200, or 500 ppm DCM for six hours per day, five days per week, for 20 months for males and 24 months for females. In addition, two groups of 30 female rats were either exposed to 500 ppm DCM for the first 12 months and to room air for the last 12 months of the study (identified as 500con in the study), or to room air for the first 12 months and 500 ppm DCM for the last 12 months of the study (identified as con500).

There was an exposure-related, statistically significant increase in the total number of benign mammary tumors in female rats exposed to 200 ppm, but not in the highest dose (500 ppm) group, although the increase was not statistically significant in any individual exposure group. The 500con and the con500 groups did not differ from the control group in the total number of benign mammary tumors in females.

There were no significant differences in any tumor type for kidney, liver, spleen, brain, salivary gland, skin, lung, or pancreas in male and female rats exposed to DCM. The results of this study offer very limited evidence of the carcinogenicity of DCM because the highest exposure concentration used in this study was not considered by the U.S. EPA (1985a) to be the MTD.

Other Studies

An inhalation study demonstrated DCM hepatic and pulmonary carcinogenicity in mice but like most of the other studies it failed to elucidate mechanisms of carcinogenesis (Kari et al. 1993, Maronpot et al. 1995). About 1,440 female B6C3F1 mice in total were exposed to 2,000 ppm DCM by inhalation for six hours per day, five days per week for various durations up to 104 weeks. Liver and lung tumors were induced in the absence of overt cytotoxicity. Compared with control animals, an eight-fold increase in the incidence of lung adenoma or carcinoma and a 13-fold increase in the total number of pulmonary adenomas or carcinomas per animal at risk were observed in DCM-exposed mice. Early exposure to DCM for as short as 26 weeks was sufficient to cause an increase in lung tumors by the end of two years. A 2.5-fold increase in the incidence of liver adenoma or carcinoma and a three-fold increase in the total number of hepatic adenomas or carcinomas per animal at risk were observed in DCM-exposed mice. Early exposure for 52 or 78 weeks was more effective than late exposure in inducing hepatocellular neoplasms which, once developed, appeared to persist after exposure was terminated (Kari et al. 1993).

The differential behavior of the target organs to varying exposure regimens suggested that the mechanisms responsible for DCM tumorigenicity could be different in the lung and the liver. Decreased replicative DNA synthesis was observed in hepatocyte labeling index at 13 weeks and pulmonary airways after one to 26 weeks of exposure. The increase in tumor induction was not associated with increased replicative DNA synthesis in liver foci or in alveolar parenchyma. The frequency and pattern of H-ras gene activation in the liver tumors and K-ras activation in the lung tumors were similar to that in the controls (Maronpot et al. 1995).

Two DCM inhalation assays using dogs, rabbits, guinea pigs, and rats showed no tumors, but were not conducted for the lifetime of the animals (Heppel et al. 1944, MacEwen et al. 1972).

Summary of Evidence of Carcinogenicity

The results of short-term testing of DCM in several bacteria have consistently provided positive evidence of mutagenic activity. These results have been obtained both with and without the addition of exogenous metabolic activation. DCM is also mutagenic to *Saccharomyces cerevisiae* yeast and *Drosophila melanogaster* fruit fly. Experiments conducted in vitro with mammalian cells have documented a dose-related increase in the occurrence of chromosome aberrations, but have provided contradictory information concerning DCM-induced SCE. The in vivo studies of clastogenic potential of DCM found some evidence that DCM induced SCE, chromosome aberrations, micronucleus, single strand breaks, and DNA damages. All of the studies conducted to date have failed to find any indication that DCM alkylates DNA, and the majority of the evidence indicates that DCM does not induce UDS. The U.S. EPA (1985a) concluded that DCM is capable of causing gene mutations and has the potential to cause such effects in humans.

Seven separate bioassays have been conducted to evaluate the carcinogenicity of DCM to animals. Of these, the NTP (1986) inhalation bioassay provided the strongest evidence that DCM is carcinogenic to rodents. On the basis of significantly elevated incidence of hepatic and pulmonary adenomas and carcinomas in male and female B6C3F1 mice, the NTP (1986) determined that there was "clear evidence" that DCM was carcinogenic to mice. The same study documented a significant increase in the incidence of various benign tumors in DCM-treated Fischer 344 rats. NTP (1986) concluded that there was "some evidence" of carcinogenicity in male rats and "clear evidence" of carcinogenicity in female rats. Relevance of carcinogenicity bioassays in mice in assessing potential health risks associated with exposure to DCM was evaluated (Liteplo et al. 1998). OSHA (1997) has determined that the NTP (1986) female mouse lung tumor response is the appropriate data set on which to base its quantitative risk assessment.

Additional evidence of carcinogenicity through ingestion of DCM in drinking water was demonstrated by a significant increase in the combined incidence of benign liver neoplastic nodules and malignant hepatocellular carcinomas in female Fischer 344 rats and male B6C3F1 mice (Serota et al. 1986a, 1986b). Increased incidences of sarcomas of the salivary gland region in male Sprague-Dawley rats (Burek et al. 1980, 1984) and pulmonary tumors in male Swiss mice (Maltoni et al. 1986, 1988), together with the increased mononuclear cell leukemia incidence in female Fischer 344 rats (NTP 1986), provide supportive evidence of the animal carcinogenicity of DCM. The question on background incidence of liver cancer in mice has been discussed (Abelson 1993, Wilson 1988).

Based on the evidence from the NTP (1986), Serota et al. (1986a, 1986b), Burek et al. (1980, 1984) and Maltoni et al. (1986, 1988) studies, the IARC (1987) and the U.S. EPA (1985a, 1987b) concluded that there is "sufficient" evidence that DCM is carcinogenic to animals. IARC (1999) concluded "The available data suggest a plausible mechanism for the development of liver and lung tumors which occur in mice." The U.S. EPA (1985a, 1987b) and IARC (1987, 1999) considered the epidemiological evidence on DCM to be "inadequate" to evaluate its carcinogenicity to humans. Consequently, DCM was placed in Group B2, as a "probable human carcinogen" by the U.S. EPA and as a 2B "possibly carcinogenic to humans" by IARC. In the U.S., CPSC (1986), FDA (1989) and OSHA (1997) all concluded that DCM is an animal carcinogen and may be carcinogenic to humans. Huff et al. (1996) concluded "DCM is a multispecies, multistrain, both-gender, multiorgan, dose-related carcinogen, clastogen, and mutagen that should be considered as a carcinogenic risk for exposed humans."

Toxicological Effects in Humans

The toxicological effects of DCM in humans have been studied in epidemiological investigations on workers whose exposures have been primarily through inhalation and may have some secondary dermal contact due to their occupation. No epidemiological studies have been conducted on ingestion of DCM through consumption of contaminated drinking water, although toxicological studies through ingestion have been performed on both rats and mice. No developmental effects were reported in workers occupationally exposed to DCM. No studies were located regarding dermal, ocular, respiratory, cardiovascular, musculoskeletal, hematological, hepatic, renal, immunological, developmental, reproductive, genotoxic, or carcinogenic effects in humans after oral exposure to DCM.

DCM intoxication in industry has been reported since the early 1900s (Collier 1936). As discussed earlier in this report, the public health concerns for DCM stem primarily from two aspects of its toxicity. First, DCM, like many other volatile organic solvents, has anesthetic action and this accounts for its depressive effects on the CNS. In addition, DCM is metabolized to CO and thereby increases COHb (Fagin et al. 1980, Soden et al. 1996) thus providing the biological potential to induce ischemic heart disease, aggravate angina pectoris, and place persons with preexisting coronary artery disease at increased risk (Kales 1997). The organic solvent properties also account for the irritation produced by contact to skin or mucous membrane. Second, animal toxicology studies have found lung and liver neoplasms related to DCM inhalation and drinking water ingestion exposures (NTP 1986, Serota et al. 1986a, 1986b).

Case reports have identified acute illness and mortality following acute inhalation or ingestion exposures to DCM (Bakinson and Jones 1985, Dhillon and Von Burg 1995, Leikin et al. 1990). The cases through inhalation were acute narcosis due to apparent respiratory over-exposure to DCM in occupational settings. The cases through ingestion were suicide attempts. Case reports in general are not appropriate for use in risk assessments as they represent acute high-dose exposures, often accidental and occupational, related to acute health outcomes. Risk assessments, such as those relating to contaminants in drinking water, by contrast, involve assessment of chronic health outcomes related to long-term low-dose environmental exposures.

Epidemiological studies provided suggestive support for the animal carcinogenicity but did not demonstrate a conclusive causal relationship between cancer deaths and occupational exposure to DCM (IARC 1999). While the results of epidemiological studies have failed to show an excess risk of lung cancer, the findings are somewhat contradictory with respect to mortality from ischemic heart disease and liver cancer. An epidemiological study reported four cases of oligospermia among workers occupationally exposed to DCM (Kelly 1988). It is uncertain whether the effect was due to DCM since the workers were also occupationally exposed to other chemicals.

Acute Toxicity

DCM exposure in an enclosed area may be dangerous since vapors may cause dizziness, headache, nausea or suffocation. Most of the toxic effects of DCM in humans have been observed after acute inhalation exposure and have resulted from its anesthetic action on the CNS, its *in vivo* conversion to CO, or its oxidation to phosgene in an open flame. CNS toxicity, characterized by headache, chest pain, fatigue, incoordination narcosis, restlessness, mental confusion and altered behavior, is the predominant effect associated with acute DCM exposures

in humans. Inhalation exposure to approximately 20,000 ppm DCM can lead to unconsciousness and death (ATSDR 1993, Dhillon and Von Burg 1995, Gerritsen and Buschmann 1960).

Direct contact with DCM causes irritation or intense burning in skin, eyes, and respiratory tract. The irritation effect may be accentuated if DCM is kept on the skin by shoes or tight clothing. The situation is severe when skin is exposed to paint remover formulations that form a layer of film. Long-term skin exposure may cause dermatitis. A 30-year-old worker in a film plate production plant inhaled DCM causing narcosis and resulting in a fall into splashed solvent. The dermal contact with concentrated DCM caused severe skin chilblains on about 40 percent of the body surface, and the worker died 28 hours after the accident (Kuzelova et al. 1975).

Inhalation as well as ingestion and skin exposure to DCM contributes to systemic toxicity (ATSDR 1993, Dhillon and Von Burg 1995). Twenty-six cases of DCM poisoning, mainly through inhalation, were reported in the literature from 1936 through October 1986. Among these, 13 acute general exposure cases, 11 acute occupational exposure cases, and two chronic occupational exposure cases were reviewed to reveal that industrial and domestic use of DCM is equally widespread (Rioux and Myers 1988). Treatment with hyperbaric oxygen worked well in three cases (Rioux and Myers 1989, Rudge 1990). Approximately 21 fatal cases of DCM poisoning have been published in the literature from 1952 to September 1999 (Baselt and Cravey 1995, Bonventre et al. 1977, Browning 1965, Chang et al. 1999, Fairfax et al. 1996, Goulle et al. 1999, Hall and Rumack 1990, 1999, Hughes and Tracey 1993, Kim et al. 1996b, Kuzelova et al. 1975, Leikin et al. 1990, Logemann and van der Smissen 1991, Manno et al. 1989, Moskowitz and Shapiro 1952, Savolainen 1989, Shinomiya and Shinomiya 1985, Stewart and Hake 1976, Tay et al. 1995, Winek et al.1981).

DCM poisoning after ingestion, including one fatality in which COHb was measured, was reported in two cases. In a suicide attempt, a 38-year-old male ingested one to two pints of paint stripper, equivalent to about 9,000 to 18,000 mg/kg of DCM. He had diverticula of the duodenal jejunal junction after six months but recovered eventually (Roberts and Marshall 1976). In another case, a 56-year-old female ingested about 300 mL of a paint stripper (Nitromors) containing predominantly DCM (Hughes and Tracey 1993). Paresthesias, somnolence, altered sleep patterns, convulsions, euphoria, and changes in cardiac rate developed after DCM ingestion. Extensive gastrointestinal ulceration and bleeding occurred after ingestion. Both patients regained consciousness after about 14 hours but the female patient died after 25 days. Her COHb level rose to a peak of 12.1 percent at about 36 hours following ingestion.

Diverse manifestations of DCM poisoning through ingestion either by accident or attempted suicide were reported recently in six cases in Taiwan including five males at the ages of 23, 28, 29, 40, and 49, and one female at the age of 27 (Chang et al. 1999). Reversible CNS depression, tachypnea, and corrosive gastrointestinal injury were the most common symptoms after ingestion DCM at volumes ranging from approximately 25 mL to 350 mL or estimated doses from 384 mg/kg to 4,794 mg/kg. Direct identification of DCM in the blood by gas chromatography-mass spectrometry was performed. An elevated COHb level was documented as 35 percent in the 49-year-old male eight hours after ingestion of 300 mL DCM, or an estimated dose of 4,286 mg/kg. COHb was 8.4 percent in the 28-year-old male 10 hours after ingestion of about 25 mL of DCM. Renal and liver failure with prolonged tissue hypoperfusion as well as acute pancreatitis occurred in the 49-year-old male, and eventually he died on day nine in the hospital. These symptoms of liver, kidney and pancreas also occurred in the 40-year-old male who ingested 350 mL DCM, however, he recovered and was discharged from the hospital on day 43. The other four patients recovered.

Accidental or intentional inhalation of high concentrations of DCM has been implicated in a number of fatalities (Bonventre et al. 1977, Browning 1965, Fairfax et al. 1996, Goulle et al. 1999, Hall and Rumack 1990, 1999, Kuzelova et al. 1975, Logemann and van der Smissen 1991, Moskowitz and Shapiro 1952, Savolainen 1989, Stewart and Hake 1976, Winek et al. 1981). Autopsy showed bleeding in the trachea, digestive tract, suprarenal, and liver of a 47-year-old man who died in the process of transferring DCM in a French factory. DCM was found in the blood at 150 mg/L, urine at 2 mg/L, gastric content at 5.6 mg/L, brain at 122 mg/kg, fat at 99 mg/kg, liver at 44 mg/kg, lung at 20 mg/kg, and kidney at 15 mg/kg (Goulle et al. 1999). In another case, DCM was detected in the liver at about 14.4 mg/dL, blood at about 51 mg/dL, serum at about 29 mg/dL, and brain at about 24.8 mg/dL (Boventre et al. 1977, Hall and Rumack 1990). The cause of death in most cases was uncertain, but myocardial infarction was reported in a fatal case of paint remover inhalation in the U.S. (Stewart and Hake 1976). CNS depression was implicated in a fatal Singapore case (Tay et al. 1995). DCM was also used as part of abused inhalant mixtures (Pryor et al. 1978). Phosgene poisoning caused by the use of chemical paint removers containing DCM in ill-ventilated rooms heated by kerosene stoves was reported (Gerritsen and Buschmann 1960).

Cases of DCM poisoning, including fatalities, during small-scale paint stripping operations were reported (Hall and Rumack 1990). Two workers died after being exposed to DCM used to remove paint in an enclosed space and three rescuers complained of dizziness and nausea (Leikin et al. 1990). Fatal exposure of a 29-year-old male painter using 10 kg stripper in a swimming pool in Germany showed blood DCM concentrations of 513 to 773 μg/mL (Logemann and van der Smissen 1991). Seven persons including one fatality inhaled a carburetor cleaner containing 43.8 percent toluene, 23.3 percent methanol, 20.5 percent DCM and 12.5 percent propane and exhibited CNS impairment, nausea, vomiting, shortness of breath, and visual symptoms (Frenia and Schauben 1993). In a double fatality case, two male workers 40 and 20 years of age died while removing the surface finish of wood floors of a squash court using a stripper containing 80 percent DCM with paraffin and toluene. Their exposure to DCM was estimated to be about 9,500 to 19,000 ppm (Fairfax et al. 1996).

Two workers involved in oleoresin extraction processes and liquid-cleaning operations died after exposure to DCM for less than one hour and up to three hours. DCM was detected at the level of 0.1 mL/500 g wet tissue in the lung, 270 mg/L in brain, and 29.8 percent in blood (Moskowitz and Shapiro 1952, Winek et al. 1981). A fatal case of accidental poisoning was reported in Korea with DCM concentrations of 252 mg/L in blood, 75 mg/kg in brain, and 30 mg/kg in heart (Kim et al. 1996b). In two acute inhalation fatalities, blood levels of DCM were as high as 168,000 ppm, comparable to the levels in the inhaled air. COHb levels were 30 percent, but these levels were not enough to account for the deaths (Manno et al. 1989, 1992). Several deaths accompanied by opisthotonos and clonic spasms in children and elderly people have been reported from the use of DCM as an anesthetic in Europe (Browning 1965).

Mahmud and Kales (1999) reported intermittent headaches in a 26-year-old male cabinet worker who had a COHb saturation of 21 percent approximately 35 minutes after leaving his work site. The lacquer thinner he used at work contained 70 percent DCM and the work area air samples had DCM levels of 300 to 500 ppm. In addition, a propane-powered fork lift inside the plant was considered a potential source of CO and the work area air samples had CO levels of 28 ppm. This case of DCM poisoning is complicated by the simultaneous exposure to ambient CO. The patient recovered once the source was removed.

Symptoms of DCM poisoning included cough, headache, lightheadedness, slowed reaction in behavior, impaired psychomotor performance, nausea, and vomiting after inhalation exposure (Cherry et al. 1981, 1983). Respiratory failure, syncope and coma may develop secondary to

CNS depression in severe exposure. Angina, myocardial infarction and cardiac arrest associated with DCM inhalation developed in one patient. Hematuria, acute tubular necrosis and exacerbation of Goodpasture's syndrome were reported (Keogh et al. 1984). A greater irregularity of response in reaction time to psychological functions was observed in 14 healthy male subjects exposed to DCM at 3,470 mg/m^3 (Gamberale et al. 1975). Most of the neurobehavioral responses to DCM appeared to be similar to the effects induced by other organic solvents and anesthetics. CNS depression has been reported in humans exposed to DCM concentrations as low as 175 ppm eight-hour TWA (Hall and Rumack 1999).

In Germany, a young male watchmaker using DCM to clean parts in a non-ventilated small room developed swelling and reddening of the skin, as well as blistering and paraesthesias affecting the hands, for seven winter seasons. The COHb concentration in blood was 13, 10, and 10.7 percent in three different samples. The dermatologic symptoms disappeared after he changed jobs permanently (Liniger and Sigrist 1994). In Israel, a 29-year-old man had acute renal failure and hepatocellular damage two days after he was exposed to DCM mainly through inhalation by working in a closed room. He recovered after treatment with hemodialysis (Horovitz and Zecler 1995). Acute renal failure with tubular necrosis, myoglobinuria, hypocomplementemia, and liver enzyme elevations was reported in a person who inhaled DCM at high concentrations over an extended period of time (Miller et al. 1985).

Metabolic conversion of DCM to CO may place persons with pre-existing coronary artery disease at increased risk. The toxic effects of CO poisoning are reversible in mild cases; however, CNS depression caused by DCM and CO has resulted in fatalities. A mean value of CO in blood of about 30 μg/mL corresponding to 12 percent COHb was obtained in human non-smoker volunteers after eight hour exposure to 500 ppm of DCM, in one case rising as high as 60 μg/mL or 24 percent COHb. The blood COHb reached five percent after an eight-hour exposure to 100 ppm of DCM. The elimination took about 24 to 26 hours to return to the original blood COHb level (Fodor and Roscovanu 1976). Elevations in COHb as high as 50 percent due to CO from DCM metabolism have been reported, and levels continue to rise for hours after exposure has ceased in some cases. The half-life of COHb is prolonged to 13 hours because of ongoing production (Hall and Rumack 1999). Nager and O'Connor (1998) reported a case of manifestation of CO poisoning generated from spray paint inhalation in a 52-year-old woman. The paint contained 31 percent DCM. Her COHb level was 11.7 percent four days after using the paint, and was reduced to 3.1 percent after treatment to relieve her symptoms.

Two cases of DCM poisoning in painters who suffered from headache, giddiness, stupor, irritability, numbness, and tingling in limbs were reported (ACGIH 1986). Multisystem disorder including nausea, headache, vomit, and anorexia was reported by a male physician after a day of stripping paints using Nitromors in a well-ventilated room with the door open (Lee 1981). Similar symptoms occurred in a healthy 25-year-old male accountant using Nitromors in a confined space for three to four hours. The patient developed skin rash as well as temporary diabetes and could not work for six months due to mental impairment (Memon and Davidson 1981, Walker and Wyke 1981). Employees exposed to DCM as part of a triacetate fiber production process had average values of COHb ranging between 1.77 percent and 4.00 percent in the nonsmoker group with a dose-related response and between 4.95 percent and 6.35 percent in the smoker group, with individually measured eight-hour TWA DCM exposures averaging up to 99 ppm (Soden et al. 1996).

Shusterman et al. (1990) reported DCM intoxication in a 35-year-old male furniture refinisher complaining of upper respiratory irritation, fatigue, and lightheadness on a daily basis after using a DCM-containing paint stripper. A comparison of exposure estimates utilizing workplace air sampling and blood COHb measurements showed a linear elevation of COHb as a function of

hours worked on the day of sampling. Noncardiogenic pulmonary edema and subsequent hyperreactive airways following exposure to DCM and its combustion product, phosgene, were reported in two separate cases, a 37-year-old male nonsmoker and a 34-year-old male using a paint remover with a heat source (Snyder et al. 1992a, 1992b).

Other case studies of DCM poisoning reported kidney and lung damage, as well as carboxyhemoglobinemia (Buie et al. 1986, Horowitz 1986, Miller et al. 1985). However, furniture stripping with DCM in a large 5,425-square-feet single-room basement by two healthy athletic non-smoking female physicians for six hours raised the blood COHb concentration to 26 percent and 40 percent, respectively, without symptoms (Langehennig et al. 1976).

Anundi et al. (1993) evaluated high exposure to organic solvents, mainly DCM, among twelve 18- to 36-year-old graffiti removers with a median age of 23 years working during daytime in underground stations in Sweden. The observed effects consisted primarily of irritative effects in the upper respiratory tract and the eyes. The eight-hour TWA exposure to DCM ranged from 18 to 1,188 mg/m^3 (about 5 to 340 ppm) with a geometric mean of 127 mg/m^3 (about 36 ppm) and a standard deviation of 3.6 mg/m^3 (about 1 ppm). Of the 12 subjects, six exceeded the Swedish Permissible Exposure Limit (PEL) of 120 mg/m^3 (about 35 ppm). The 15-minute samples ranged from six to 5,315 mg/m^3 (about two to 1,520 ppm) with a geometric mean of 400 mg/m^3 and a standard deviation of 5.59 mg/m^3. Most short-term samples exceeded the Swedish short-term exposure limit (STEL) of 300 mg/m^3.

Experimental studies on human exposure to high concentrations of DCM of more than 300 ppm for more than one hour suggest that DCM may impair vision and hearing (Forster et al. 1974). Breathing DCM at 800 ppm for short periods may impair reactiveness, steadiness, and precise hand movements. Breathing 300 ppm or greater for about three to four hours can depress the CNS with symptoms of lightheadedness, decreased hand-eye coordination and auditory signal detection, and altered behavior. Inhalation of about 490 ppm DCM may produce eye irritation and cornea damage. In most cases, the neurological, behavioral, and psychophysiological effects disappear shortly after exposure ends (Fodor and Winneke 1971, Kelly 1988, Putz et al. 1976, Winneke and Fodor 1976, Winneke 1974, 1981, 1982). Neurotoxicity can be permanent (Hall and Rumack 1999) and is thought to be due to both a direct nonspecific CNS depression action of DCM and indirect effect of CO (Winek et al. 1981).

DCM concentrations in excess of 50,000 ppm are thought to be immediately life threatening due to asphyxiation (Hall and Rumack 1999). The lowest toxic airborne concentration in humans has been estimated to be 500 ppm (HSDB 1999). Exposure to 200 ppm DCM produced a 6.8 percent COHb level (DiVincenzo and Kaplan 1981a). A COHb level of 3.4 percent was produced with inhalation of about 500 ppm DCM (Stewart et al. 1977). Breathing 1,000 ppm DCM for two hours produced greater than 7.9 percent COHb (HSDB 1999). A COHb level of 50 percent was found in an unconscious person who had been using a DCM-based paint stripper and COHb remained elevated for at least 24 hours after acute DCM exposure (Stewart et al. 1972b).

Exposure to DCM is likely to coexist with other toxic substances in an occupational environment. DCM is a common solvent carrier in pesticide applications and multiple chemical hypersensitivity has been reported in 17 workers exposed to insecticides and solvents containing DCM (Cone and Sult 1992). Firefighters are routinely exposed to complex mixtures used in building materials including DCM (Golden et al. 1995). Co-exposure to CO and DCM was reported to generate neuropathy (Dick 1992).

Subchronic and Chronic Toxicity

There have been relatively few reports of subchronic or chronic toxic effects resulting from human exposure to DCM. Chronic exposure to DCM in humans causes CNS depression. Another main effect of chronic exposure to DCM is an increase in the COHb level in the blood (Adams and Erickson 1976). In studies in which volunteers were exposed to DCM by inhalation over a five-week period, for various lengths of time and at different concentrations, blood COHb levels increased with exposure, but there was no evidence that the two effects of DCM-induced CNS depression and elevated blood COHb levels are associated with each other. Chronic DCM exposure has been reported to result in kidney damage (Miller et al. 1985) and chronic renal hypertension (Loyke 1973).

There are several case reports of toxicity resulting from occupational exposure to DCM, but concurrent exposure to other chemicals make interpretation of these studies difficult. Reported effects included encephalopathy, eclampsia, and bilateral temporal lobe degeneration (Barrowcliff 1978, Weiss 1967). Three-year occupational exposure to 300 to 1,000 ppm DCM might be the cause for memory loss with intellectual impairment and balance disturbance in a 58-year old man. Bilateral temporal lobe degeneration appeared with persistent formation of endogenous blood CO from the adsorbed DCM (Barrowcliff and Knell 1979). A chemist after a year's exposure to DCM developed toxic brain encephalosis with acoustical and optical delusions and hallucinations (ACGIH 1986). Nervous system disorders were reported in 30 percent of workers chronically exposed to airborne levels of 1.8 to 9.8 ppm of DCM (Kashin et al. 1980). A Czech study reported neurological complaints in workers exposed to DCM at airborne levels of 500 ppm or less (Kuzelova and Vlasak 1966).

Kelly (1988) documented widespread complaints of CNS dysfunction among auto parts workers exposed to large quantities of DCM mainly by inhalation. CNS effects reported included dizziness, loss of consciousness, memory loss, depression, and personality change. In Sweden, Anundi et al. (1993) studied 12 male graffiti removers, with a median age of 23 years and work history from three months to 4.5 years, for exposures to organic solvents. Six of the 12 subjects were exposed to DCM levels exceeding the Swedish standard of 120 ppm and the concentrations for the eight-hour TWA ranged from 18 to 1,188 ppm. A significant increase in eye and upper respiratory tract irritation was noted among the exposed when compared with the referents.

Chronic neurotoxicity of DCM exposure has been reviewed (ECETOC 1996). No firm evidence of lasting CNS effects in a total of 1,758 retired airline mechanics with long-term exposure to DCM was reported in a cohort survey (Lash et al. 1991). Friedlander et al. (1978) reported that no increase in death occurred among workers exposed to TWA of 30 to 120 ppm DCM for over 30 years. Ott et al. (1983b) reported that fiber production workers exposed to 140 to 475 ppm DCM for at least three months did not have a significant increase in mortality. Hearne and Pifer (1999) reviewed two overlapping cohorts of photographic film base manufacturing employees of Eastman Kodak Company. The first group of 1,311 men was exposed to DCM at a mean of 39 ppm eight-hour TWA for 17 years with a median time of 34 years, the second group of 1,013 men was exposed to DCM at an average of 26 ppm for 24 years with a median time of 35 years. No statistically significant increases in mortality were observed for all causes of death evaluated.

Ott et al. (1983a, 1983c) studied the mortality and clinical laboratory evaluations of 1,271 workers occupationally exposed to DCM at an average of 475 ppm, equivalent to 1,650 mg/m^3, in a triacetate fibers plant in Rock Hill, South Carolina. Six serum constituents that had the potential to detect possible liver injury in relation to DCM exposures were examined. A dose-related rise in serum bilirubin was observed for both men and women but the relationship of this

endpoint to liver damage is unclear. Women in the subgroup exposed to 475 ppm DCM showed an increase in red cell counts, hemoglobin, and hematocrit, but men did not. These finding are suggestive of a hematopoietic effect. On the basis of the epidemiological studies by Ott and coworkers, the U.S. EPA (1983) estimated that chronic inhalation exposures of up to about 100 ppm, equivalent to 347 mg/m^3, might represent a no-observed-effect level (NOEL) for humans.

Later in 1993, Soden used the same group of employees (n = 1,271) at the Rock Hill plant exposed to an average of 475 ppm eight-hour TWA of DCM for greater than 10 years (Lanes et al. 1990, Ott et al. 1983a, 1983c) to compare to a similar non-exposed group of employees. It appeared that chronic exposure to DCM produced no differences in blood chemistry tests including SGOT and SGPT (two standard liver function tests), total bilirubin, and hematocrit among the two groups (Soden 1993).

Reproductive and Developmental Toxicity

Sullivan et al. (1993) reviewed the potential of DCM to be toxic to the reproductive system. Limited data reported in the Occupational Safety and Health Reporter indicated a greater risk of sterility in male workers exposed to DCM (OSHA 1997). Detectable levels of DCM were found in the blood, breast milk, embryonal, fetal and placental tissues of nursing women exposed to approximately 24 ppm DCM in a Russian rubber product plant (Byczkowski et al. 1994, Vozovaya et al. 1974). Both DCM and its metabolite CO cross the placenta and have been found in fetal tissue of pregnant women occupationally exposed to high levels of DCM (Hake et al. 1974, Schwetz et al. 1975, Tabacova 1986). The biological effects of CO in the pregnant woman, fetus, and newborn infant have been reported (Longo 1977).

Increased spontaneous abortion rates of borderline statistical significance were reported in female workers exposed to DCM and other solvents during the first trimester of pregnancy in two European studies. The first one, with a relative risk of 1.3 and a confidence interval of 0.89 to 1.91, was in a total of 1,160 pregnancies in 782 laboratory employees at the University of Gothenburg between 1968 and 1979 (Axelsson et al. 1984). The second one, with an odds ratio of 2.0 and a 95 percent confidence interval of 1.0 to 5.7 ($p = 0.06$), was among 174 Finnish pharmaceutical workers during 1973 or 1975 to 1980. The frequency of exposure to DCM was 11/38 in cases and 17/119 in controls (Taskinen et al. 1986).

Increased spontaneous abortion risk was reported to be related to occupational exposure to solvents in laboratory work of both parents in a Finnish study (Lindbohm et al. 1984). Paternal exposure to solvents used in manufacturing of rubber products including DCM and 1,1,1-trichloroethane was associated with an increased relative risk of spontaneous abortion in another Finnish study. An odds ratio of 1.9 with 95 percent confidence interval of 1.2 to 2.8 among 26 spontaneous abortions in 180 pregnancies was reported (Lindbohm et al. 1991). No significant adverse effects of DCM exposure on birthweight were found in Monroe County, New York (Bell et al. 1991).

Occupational exposure to DCM through inhalation has been implicated in four cases of oligospermia among workers in Cincinnati (Kelly 1988). Over a two-year period, 34 male workers from an auto parts factory were evaluated for health complaints, primarily associated with CNS dysfunction. Two of the eight men complained of chronic pain in the groin area involving prostate and genitals. These two men exhibited bilateral testicular atrophy, and had clinical history of infertility. These two, and additional four workers who provided semen for analysis, were found to have sperm counts in the “sub-fertile or infertile range”, concluded by the

author. Abnormalities were also observed with respect to sperm motility and morphology. All eight individuals were exposed to large quantities of liquid DCM, with duration of exposure to DCM varied from 0.4 to 2.9 years overall. For the four workers with abnormal sperm counts, the range was 1.4 to 2.9 years.

To confirm DCM exposure, blood COHb determinations, made four to 24 hours after the last known exposure to DCM, revealed COHb levels that ranged from 1.2 to 17.3 percent as compared to 8 to 10 percent in smokers (Kelly 1988). NIOSH determined that in the areas where bonding operations took place, the average concentration of DCM was 68 ppm, with a range of 3.3 to 154.4 ppm. However, it is not known whether these levels are representative of conditions over the entire period of employment. Kelly (1988) concluded that although it is not possible to rule out other explanations for the oligospermia in these workers, several factors are consistent with the hypothesis that DCM was a causative agent. These include the relatively high concentration of DCM in the workplace, elevated blood COHb levels, and minimal exposure to other potential toxic substances.

In a study of 11 furniture strippers exposed to a mean DCM concentration of 122 ppm with a mean blood COHb concentration of 5.8 percent, none of the men had oligospermia with a mean sperm density of 54 million per mL (Wells et al. 1989). In a prospective study, 50 men who were exposed to solvents including DCM and fuels by working on aircraft maintenance at an installation at Hill Air Force Base and eight unexposed men were compared for male reproductive effects before the first exposure and at 15 and 30 weeks after exposures had begun. For all solvents and fuels, all industrial hygiene measures were below 6 ppm; which is less than the OSHA standard. Sheet metal workers had the highest mean breath levels for both total solvents (24 ppb) and fuels (28.3 ppb). For most sperm measures, mean values remained in the normal range throughout the 30 weeks of exposure. However, the paint shop group had a significant decline in sperm motility of 19.5 percent at 30 weeks (Lemasters et al. 1999b). Genotoxicity evaluations of the same groups of workers are described in the next section (Lemasters et al. 1997, 1999a).

Genotoxicity

Genotoxicity of DCM alone in humans has not been reported (Broome and Sivak 1988, ECETOC 1987, IARC 1986, 1987, Strobel and Grummt 1987, U.S. EPA 1985a). Lymphocytes collected from 46 workers occupationally exposed to styrene and DCM and 23 controls were measured for frequencies of HPRT mutants, chromosomal aberrations with and without gaps, aberrant cells, micronuclei, SCEs, and cells with high frequencies of SCEs. TWA measurement values for styrene and DCM exposure during an eight-hour working day were 70 mg/m^3 (about 17 ppm) with a range from 0 to 598 mg/m^3, and 108 mg/m^3 (about 31 ppm) with a range from 0 to 742 mg/m^3, respectively. All cytogenetic parameters were significantly enhanced ($P < 0.0001$, one-sided) in exposed workers, however, no definite conclusions could be drawn due to the lack of appropriate control data. The DCM TWA value was positively correlated with the frequencies of chromosomal aberrations with gaps and aberrant cells (Tates et al. 1994).

Genotoxic changes, especially small increases in SCEs, were reported in aircraft maintenance personnel after low-level exposure to fuel and solvents. Air sampling, expired breath, blood, and urine analysis for jet fuel JP-4, 1,1,1-trichloroethane, methyl ethyl ketone, xylenes, toluene and DCM, in conjunction with SCE and micronuclei frequency were compared for 58 newly hired men aged between 18 and 50, working on equipment operation and maintenance at Hill Air Force Base prior to exposure and over 30 weeks, and for eight unexposed men as the control. Mean

exposure levels were all below 6 ppm, less than the OSHA standard. Expired breath levels were all below 25 ppb. A statistically significant increase in SCE frequency occurred after 30 weeks of exposure for sheet metal workers ($P = 0.003$) and for painters ($P = 0.05$). The micronuclei frequency in the sheet metal workers initially showed a statistically significant increase by 15 weeks, but decreased by 30 weeks. DCM in breath in eight subjects ranged from 0 to 2 ppb, and DCM in blood measured as COHb in six subjects ranged from 1.3 to 2.2 mg/mL. However, it is unclear that these genetic changes are due to DCM exposure (Lemasters et al. 1997, 1999a).

Carcinogenicity

IARC (1999) reviewed the available seven cohort studies and three case control studies on DCM carcinogenicity in humans. Sporadic and weak associations were reported for cancers of the pancreas, liver and biliary passages, breast, and brain in epidemiological studies of DCM inhalation exposure. These studies were also reviewed by Dell et al. (1999). Liver and biliary tract cancers were found in one occupational mortality study (Lanes et al. 1990); however, this finding was not identified in other occupational inhalation studies (Bond et al. 1990, Gibbs et al. 1992, Hearne et al.1990), nor was it confirmed in an extended mortality follow-up of the same cohort (Lanes et al. 1993). Excess pancreatic cancer has been suggested (i.e., elevated but was not statistically significant) in one study of DCM inhalation exposure (Hearne et al. 1987) but not confirmed when mortality follow-up was extended (Hearne et al. 1990). A dose-response relationship between DCM exposure and astrocytic brain cancer was reported in a case-control study of electronics workers (Heineman et al. 1994), although this finding has been viewed as resulting from a flawed exposure calculation and thus lacking in scientific merit (Norman 1996). A non-dose-response relationship between DCM and female breast cancer was reported in a 24-state mortality study (Cantor et al. 1995); non-dose-response relationships are not considered to be compelling epidemiological evidence.

In contrast to the sufficient evidence of animal carcinogenicity, IARC (1999) concluded that: "While seven cohort studies and three case-control studies have examined the risk of cancer associated with exposure to DCM, for no type of cancer was there a consistent elevation of risk across these studies to make a causal interpretation credible, and epidemiological evidence for carcinogenicity in humans was thus judged inadequate." U.S. EPA (1997a) considered the human carcinogenicity data on DCM inadequate for classification of the chemical since none of the few studies of chemical factory workers exposed to DCM showed an excess of cancers (Friedlander et al. 1978, Hearne and Friedlander 1981, Ott et al. 1983, Tomenson et al. 1997). FDA (1989) finds that the sensitivity of the human studies is insufficient to rule out the possibility that DCM can cause cancer in humans as inferred from the rodent studies. OSHA (1997) believes that the non-positive epidemiology data on human cancer are of insufficient power to rule out the cancer risk estimates derived from the animal data. Similar conclusions are made by Dell et al. (1999) "…it appears likely that risks associated with DCM exposure… are small and limited to rare cancers."

Major Epidemiological Studies in Humans

Case Control Studies

The major occupational case control studies in epidemiology to determine the health effects of DCM exposure through inhalation follow.

a) Heineman et al. (1994)

A job-exposure matrix case-control study among electronic equipment production and repair workers by the NCI (Heineman et al. 1994) was conducted to examine the association between brain cancer and exposure to a group of six chlorinated aliphatic hydrocarbons including DCM. Associations of occupational exposure to DCM, in terms of estimated intensity, probability and duration of exposure, and mortality from astrocytic brain cancer in white males were observed. The study consisted of 300 astrocytic brain cancer cases and 320 controls in southern Louisiana, northern New Jersey and Philadelphia, Pennsylvania. Risk of these brain tumors increased with probability and average intensity of exposure, and with duration of employment in jobs considered exposed to DCM. Among the six chlorinated aliphatic hydrocarbons evaluated, the association was strongest for DCM. There are many limitations to this type of study, but the authors believed that the trends and consistency of the associations could not be explained by chance alone. Both OSHA and NIOSH agreed that the Heineman et al. (1994) study was well conducted and the results strongly suggest a possible association (OSHA 1997).

b) Cantor et al. (1995)

Mortality records from 24 states gathered from 1984 to 1989 were used to develop leads to workplace exposure as possible breast cancer risk factors with a job-exposure matrix case control approach (Cantor et al. 1995). After adjusting for socioeconomic status, suggestive associations for probability and level of exposure among whites and blacks were found for DCM using 33,509 cases and 117,794 controls. However, simultaneous exposure to many solvents is common, which is a major limitation of the study. DCM was a common exposure in three breast cancer deaths in a cohort study of aircraft maintenance employees (Spirtas et al. 1991, Stewart et al. 1991).

Cohort Studies

The major occupational cohorts studied epidemiologically to determine the health effects of DCM inhalation follow. Significant findings from these studies are presented in Table 8. The study detailed below is presented in a uniform spreadsheet-like format for ease of comparing one study with another.

a) The Eastman Kodak cohort – photographic film manufacture

1) Friedlander et al. (1978)

Method: Retrospective cohort mortality study of Eastman Kodak photographic film manufacturing plant workers. Mortality among a cohort of male DCM-exposed workers, established in 1964, was compared to mortality in three groups of non-concurrent controls: Kodak Park workers, New York State residents exclusive of New York City, and residents of the U.S. Mortality follow-up was completed to 1976.

Exposure measures: Over 30 years of industrial hygiene measures indicated that DCM work area exposures were likely to be stable and likely to range from 33 ppm to 118 ppm, TWA. No worker personal exposure measures were available.

Range of outcome variables considered: Malignant neoplasms, circulatory disease, and *all cause* mortality.

Sample size: A total of 751 hourly male workers employed in 1964 were followed. A sub-group of 252 workers with more than 20 years of exposure were identified and analyzed separately.

Control over extraneous variables: Mortality was age-standardized and the sample restricted to males. Other controls were not addressed; however the use of an occupational cohort control was an attempt to control for personal and lifestyle risk factors.

Cancer latency: There was a 13-year follow-up for the more than 20-year exposure sub-group.

Significant findings: None. No increased risk for malignancy, circulatory disease, or total mortality in the DCM exposed population.

Significant study limitations: Only 13-years of mortality follow-up. Small sample size (n = 751).

Friedlander et al. (1978) studied the mortality experience of a population of males occupationally exposed to DCM through their employment at an Eastman Kodak Plant. Exposure data were obtained from work area and personal samplers over a 17-year period. The authors estimated that the TWA concentration of DCM in these areas ranged from 30 to 120 ppm, with maximum measured concentration of 350 ppm in 1959. In the first phase of this work, Friedlander et al. (1978) conducted a proportionate mortality study in which exposed employees who died during the period 1956 to 1976 were compared to those without history of DCM exposure who were designated as controls. Of the 334 deaths that occurred in the study population over the 20-year period in question, there were no significant differences between the number of observed and expected deaths for any specific cancer site. The second study (Friedlander et al. 1978) reported the results of a historical cohort mortality study of 751 male hourly employees of the Eastman Kodak plant from 1964 to 1976. Cause-specific mortality among the study group was compared with that of unexposed male hourly employees, as well as with the mortality experience of New York State males (exclusive of New York City) of the same age group. Death rates from all causes were not significantly different among exposed workers and internal controls, although the standard mortality ratio of exposed workers was significantly ($p < 0.01$) lower than the New York State controls. The number of observed deaths due to malignant neoplasms and circulatory disease were also significantly less ($p < 0.01$) than expected among the exposed population compared to New York State controls.

2) Hearne and Friedlander (1981)

Method: Retrospective cohort mortality study which is an extension of the Eastman Kodak male photographic film manufacturing plant worker cohort described in Friedlander et al. (1978). Mortality follow-up was extended from 1976 to 1980.

Exposure measures: Not mentioned. However, this is the same cohort described in Friedlander et al. (1978).

Range of outcome variables considered: Malignant neoplasms, circulatory disease, respiratory disease, accidents, and mortality from all causes.

Sample size: There were 59 observed deaths based on 11,445 person-years of mortality follow-up.

Control over extraneous variables: Mortality rates were age standardized and the sample was restricted to males. Other controls were not mentioned.

Cancer latency: There was 17 years of latency for the cohort sub-set with more than 20 years of DCM exposure.

Significant findings: None. The prior conclusion (Friedlander et al. 1987) of no relationship between DCM exposure and mortality was supported by the four additional years of mortality follow-up.

Significant study limitations: Limited mortality follow-up, small sample size.

An update of the historical cohort study of Friedlander et al. (1978), in which the follow-up period was extended through 1980, produced a similar conclusion (Hearne and Friedlander 1981).

3) Hearne et al. (1987)

Method: Retrospective cohort mortality study which is an extension of the Eastman Kodak photographic film manufacturing plant worker cohort first described in Friedlander et al. (1978) with mortality ascertainment extended to 1980 by Hearne and Friedlander (1981). This paper extends mortality ascertainment to 1984.

Exposure measures: Exposures were based on a career exposure index derived from job histories and industrial hygiene measures. No personal samples were available.

Range of outcome variables considered: Lung cancer, liver cancer, ischemic heart disease, total malignancies, and total deaths were considered.

Sample size: A cohort of 1,013 hourly DCM-exposed workers.

Control over extraneous variables: Age and sex.

Cancer latency: Median latency 30 years.

Significant findings: No significant excesses for deaths due to cancer or cardiovascular disease among workers with chronic DCM exposure. There was a suggestive excess (not statistically significant) of pancreatic malignancy.

Significant study limitations: This study is enhanced over the previous analysis of the same worker cohort through increased mortality follow-up and a slightly larger sample size.

A further update of the mortality experience of Eastman Kodak employees has been published by Hearne et al. (1987). The study cohort included all 1,013 full time hourly male workers who had been employed in areas of the Kodak plant in which DCM was used from 1964 to 1970. There were no significant differences in the number of observed and expected deaths for lung cancer, liver cancer, or ischemic heat disease between the cohort and control groups. The total number of deaths from all causes was significantly different from that in the Kodak control group. However, there were eight deaths due to pancreatic cancer among exposed workers, compared to about 3.1 deaths expected in the control groups. This increase was not statistically significant when tested at $p = 0.001$, and there was no dose-response relation between exposure to DCM and the development of pancreatic cancer. Individuals that died from pancreatic cancer were potentially exposed to the suspected carcinogens 1,2-dichloropropane and 1,2-dichloroethane. Further, Hearne et al. (1987) reported that they have unpublished data which indicated an association between DCM exposure with consideration of latency and the development of this cancer.

The report of Hearne et al. (1987) was criticized by Mirer et al. (1988), on the interpretation of pancreatic cancer risk. Specifically, Mirer et al. (1988) noted that the eight observed deaths from

cancer of the pancreas represented a 2.5-fold excess risk which was statistically significant ($p = 0.034$, two-tailed Poisson test) "by the criteria conventionally applied to cohort mortality studies."

In response to these criticisms, Hearne et al. (1988) agreed that mortality due to pancreatic cancer among exposed workers was significantly different from controls if tested at $p < 0.05$. However, since no new cases of pancreatic cancer had been reported through "mid-1987", the current number of expected deaths from this cancer increased to 3.9, which is no longer significantly different ($p = 0.09$) from eight observed deaths among exposed workers.

4) Hearne et al. (1990)

Method: Retrospective cohort mortality study of Kodak film manufacturing plant workers. The cohort was established for persons who had at least one year of DCM exposure and who were working between 1964 and 1970. Mortality follow-up was extended to 1988. Workers comparisons were made to the general New York State population exclusive of New York City and to other industrial referents (other Kodak employees).

Exposure measures: DCM industrial hygiene work area exposures measured from 140 to 475 ppm (eight-hour TWA).

Range of outcome variables considered: Lung cancer, pancreatic cancer, liver cancer, and ischemic heart disease.

Sample size: 1,013 hourly photographic film manufacturing plant workers.

Control over extraneous variables: Age, sex, and calendar year period for the total sample, and smoking data on 3/4ths of the sample. For the 3/4ths sample, cigarette smoking in the study cohort was similar to that in the general population.

Cancer latency: Median 33 years.

Significant findings: No significant findings for the health outcomes of interest (lung cancer, liver cancer, or ischemic heart disease) which were tested due to their presumptive relationship to DCM exposure. A previous finding from the same cohort with three years less of mortality follow-up reported a suggestive pancreatic cancer elevation (Hearne et al. 1987); however, this elevation was not supported with the additional mortality follow-up.

Significant study limitations: Study enhanced over previous analysis of the same worker cohort through increased mortality follow-up.

In the Friedlander et al. (1978) study, exposures were low, but the data provided some suggestion of an increased incidence of pancreatic tumors. This study was recently updated to include a larger cohort, followed through 1984, and an investigation of possible confounding factors (Hearne et al. 1986, 1987). A nonsignificant excess in pancreatic cancer deaths was observed, which was interpreted by U.S. EPA (1987a) as neither clear evidence of carcinogenicity in humans, nor evidence of noncarcinogenicity.

b) The Hoechst Celanese cohorts at Rock Hill, South Carolina, and Narrows, Virginia - cellulose triacetate manufacture

1) Ott et al. (1983a-1983e). (This study is presented in five sequential journal articles.)

Method: Retrospective cohort mortality study. Workers were studied who had more than three months of exposure from January 1, 1954 to January 1, 1977 and mortality ascertainment was completed to June 1977. Worker mortality was compared to U.S. mortality.

Exposure measures: Industrial hygiene work area monitoring results ranging from 140 ppm to a high of 475 ppm of DCM. Concomitant exposures to methanol at 0.1 of the level of DCM, and acetone exposures from 100 to over 1,000 ppm also occurred. No personal measures such as cigarette smoking or alcohol use were available.

Range of outcome variables considered: Hematopoietic and circulatory system outcomes were studied, specifically ischemic heart disease and malignant neoplasms.

Sample size: 1,271 persons in the exposed cohort, 948 persons unexposed.

Control over extraneous variables: The comparison plant had the same design except without DCM use. Control was introduced on sex, age at entry, years since entry, and length of employment.

Cancer latency: 23 years maximum.

Significant findings (presented in the different journal articles):
Cancer mortality: None.
Clinical laboratory: Increased red cell counts, hemoglobin, and hematocrit observed in women but not men. Increased bilirubin observed in both sexes.
Electrocardiographic monitoring: None.
Metabolism and oxygen half-saturation: Dose-related increases in the level of COHb and alveolar CO and a corresponding decrease in the oxygen half-saturation pressure.

Significant study limitations**:** Small sample size and concomitant exposure to other solvents. However, this study overcomes some of the limitations of previous studies in that it includes both sexes and the DCM exposure levels are greater.

A series of five reports by Ott et al. (1983a-1983e) chronicled the results of studies of worker health at two synthetic fiber production plants owned by Dow Chemical Company. At one plant, the principal solvent in use was acetone. Measurements made during a five-month period in two principal work areas documented TWA concentrations of DCM of 140 to 475 ppm, methanol of 10 to 50 ppm, and acetone at both plants of 100 to 1,000 ppm.

For the retrospective mortality study (Ott et al. 1983a), the cohort was composed of male and female employees who had worked a minimum of three months in the preparation or extrusion areas of either of the plants during the period 1954 to 1977. Ultimately, 1,271 employees from the DCM plant and 948 employees from the acetone-only plant were selected. Among exposed workers, no excess mortality was found due to cardiac disease, site-specific malignant neoplasms, or total malignant neoplasms.

Clinical studies however, found that women exposed to DCM had elevated erythrocyte counts, as well as elevated hemoglobin and hematocrit levels. DCM-exposed workers of both sexes had lower than normal levels of serum bilirubin, but electrocardiographic monitoring of workers found no significant differences between DCM-exposed workers and the reference population (Ott et al. 1983b-1983e).

Reviews of these reports by U.S. EPA (1985a) and DHS (1989) have noted that the studies of Ott and coworkers were limited in their ability to detect exposure-related increases in cancer, due in part to the small number of deaths in the cohort of 54 total deaths, seven of which were from cancer. In addition, relatively few workers had been followed for 17.5 years which was the maximum possible, and the majority were followed for considerably shorter periods of time. As such, the follow-up periods were probably insufficient to detect any exposure-related increases in cancer.

2) Lanes et al. (1990)

Method: Retrospective cohort mortality study of cellulose fiber production workers established for workers between January 1, 1954 and January 1, 1977 who had at least three months of DCM exposure. Mortality follow-up was completed to September 1, 1986. Worker deaths were compared to mortality in York County, South Carolina.

Exposure measures: Industrial hygiene work area measures of DCM ranged from below detection to 1,700 ppm. There were concomitant exposures to methanol at 0.1 of DCM and also exposures to acetone.

Range of outcome variables considered: All causes, malignant neoplasms, accidents, hypertension without heart disease, cerebrovascular disease, ischemic heart disease, non-malignant respiratory disease, and suicide were studied.

Sample size: There were 1,271 cellulose fiber production workers employed in the preparation and extrusion areas, and 122 deaths.

Control over extraneous variables: Age, sex, and race.

Cancer latency: 32 years maximum.

Significant findings: Strong association between DCM exposure and liver and biliary tract cancers (SMR = 5.75) was reported. There was no support for an association between DCM exposure and pancreatic cancer. Accidents, not a hypothesized association, were also elevated (SMR = 1.64).

Significant study limitations: Small sample size, limited mortality follow-up, and concomitant exposure to other solvents.

3) Lanes et al. (1993)

Method: Retrospective occupational cohort mortality study, with cohort established from January 1, 1954 through January 1, 1997. This is the same cohort described in Lanes et al. (1990) with mortality follow-up extended four more years to 1990. Worker mortality compared to York County, South Carolina.

Exposure measures: Industrial hygiene work area characterization of DCM exposures measured from 140 to 745 ppm eight-hour TWA.

Range of outcome variables considered: Liver cancer, biliary tract cancer, pancreatic cancer, and ischemic heart disease.

Sample size: 1,271 production workers with more than three months of DCM exposure.

Control over extraneous variables: Not mentioned.

Cancer latency: 36 years, four more years than Lanes et al. (1990).

Significant findings: No excess deaths from ischemic heart disease. The excess deaths for liver cancer and biliary tract cancer observed in the shorter follow-up period (Lanes at al. 1990) were not elevated when mortality follow-up was extended.

Significant study limitations: Small sample size and concomitant exposures to methanol and acetone.

c) The Amcelle cohort at Cumberland, Allegany County, Maryland – cellulose triacetate manufacture.

1) Gibbs et al. (1996)

Method: Retrospective cohort mortality study. Worker cohort established from January 1, 1970 to 1981 (plant closure). Mortality follow-up was completed to December 31, 1987. Mortality comparisons were made to U.S., State of Maryland, and also to Allegany County, Maryland (the primary comparison).

Exposure measures: Industrial hygiene work area characterization of DCM measured from 300 ppm to 1,250 ppm. Two exposure ranges: 350 to 700 ppm and 50 to 100 ppm.

Range of outcome variables considered: Biliary tract cancer, liver cancer, pancreatic cancer, and lung cancer were studied.

Sample size: There were 3,211 cellulose-fiber production workers with more than three months of employment.

Control over extraneous variables: Control over SES indicators is assumed by using Allegany County, Maryland, as a comparison.

Cancer latency: Latency is 38 years maximum.

Significant findings: There is a deficit in *all causes* of death. There was no excess of biliary tract cancer mortality, no excess of liver cancer mortality, and no excess of pancreatic cancer mortality. However, men with more than 20 years of employment and high DCM exposure experienced elevated prostate cancer and women with more than 20 years of employment and low DCM exposure experienced elevated cervical cancer (these findings are difficult to interpret and are not consistent with those of other studies).

Significant study limitations: Limited mortality follow-up and concomitant exposures to finishes, lubricating oils, pigments, dyes, and dusts.

d) The Brantham United Kingdom cohort - photographic film manufacture

1) Tomenson et al. (1997)

Method: Retrospective cohort study of photographic film employees. The cohort was established from workers employed from 1946 to 1988. Mortality follow-up was completed to December 1994. Worker death rates were compared to national rates (England and Wales) and to local rates.

Exposure measures: Four exposure periods were established: before 1960, 1960 to 1969, 1970 to 1979, and 1980 to 1989. Within each period separate exposure measures were established for each of 20 distinct work groups based on area monitoring. Lifetime cumulative exposure measures were then calculated for each employee. Cumulative exposure categories were zero, zero to 399, 400 to 799, and $\geq$ 800 ppm-years of DCM exposures. Mean duration of worker exposure was nine years.

Range of outcome variables considered: Liver cancer, biliary tract cancer, and cardiovascular disease were studied.

Sample size: 1,785 male photographic film production workers.

Control over extraneous variables: Limited to males, control on age, cigarette smoking was curtailed in the workplace, other controls not mentioned.

Cancer latency: 48 years maximum.

Significant findings: Substantially reduced mortality rates for all causes, for all cancers, and for liver cancer, biliary tract cancer, lung cancer, pancreatic cancer, and for cardiovascular disease.

Significant study limitations: Small sample size, limited to males.

Tomenson et al. (1997) investigated patterns of mortality of workers exposed to DCM employed at a plant producing cellulose triacetate film base at Brantham in the United Kingdom, in particular, mortality from liver and biliary tract cancer, lung cancer, pancreatic cancer, and cardiovascular disease. A total of 1,473 male subjects with a record of employment at the film factory from 1946 to 1988 worked in jobs that entailed exposure to DCM. The mean duration of exposure was nine years at 19 ppm of eight-hour TWA. As of December 31, 1994, 334 deaths were identified. Mortalities for the cohort were compared with national and local rates and expressed as standardized mortality ratios (SMR). There were no significant differences in the number of observed and expected deaths for lung cancer, liver cancer, or ischemic heat disease between the cohort and control groups.

e) Studies of solvent exposures that included exposures to DCM, but for which DCM was not the primary focus of the study

1) Wilcosky and Tyroler (1983) The rubber industry cohort

Method: Retrospective cohort mortality study of 1,282 white male rubber and tire manufacturing plant workers who were between 40 and 84 years old as of January 1, 1964 and who were either currently working or who had retired following completion of 10 years of employment. Exposed worker mortality was compared to mortality of non-exposed workers.

Exposure measures: Exposure measures were derived from job histories, which specified work in a job where any of 25 solvents were authorized to be used. Neither actual industrial hygiene measures were involved, nor was verification of solvent use attempted.

Range of outcome variables considered: The focus of analysis was on cardiovascular disease and ischemic heart disease.

Sample size: There were 1,282 white males and 289 cases of cardiovascular disease mortality.

Control over extraneous variables: DCM could not be evaluated separately since it was used only when phenol was present. The study was restricted to white males. Hypertension and serum cholesterol is not controlled.

Cancer latency: Maximum of 15 years.

Significant findings: DCM exposure showed a strong positive association with ischemic heart disease (rate ratio = 5.6, $p < 0.05$) but only in the youngest age group.

Significant study limitations: Small sample size, confounding phenol exposure.

2) Spirtas et al. (1991) Aircraft maintenance cohort

Method: Retrospective cohort mortality study of (predominantly white) civilian aircraft maintenance workers at a Utah Air Force Base who had one or more years of work exposure between January 1, 1952 and December 31, 1956 to a variety of solvents, one of which was DCM. Mortality follow-up was to December 31, 1982 (25 years). Worker deaths were compared to Utah white male mortality.

Exposure measures: Based on work records, 43,000 job and organization combinations were used to index exposures to 28 solvent exposure categories.

Range of outcome variables considered: Sixty-two cause-of-death categories based on International Classification of Diseases (ICD), 8th revision, plus *all causes* of death.

Sample size: There were 14,457 workers and 1,508 deaths.

Control over extraneous variables: Age, sex, and calendar year period were controlled. There was indirect control over some SES and lifestyle variables by virtue of using a Utah worker population and a Utah comparison population. Individual smoking histories, religion, and alcohol consumption variables were not available.

Cancer latency: Maximum 30 years.

Significant findings: Elevated multiple myeloma among men exposed to DCM (SMR = 574, 95 percent confidence interval = 156 - 1,469). Among men, elevated asthma was related to DCM exposure. Among men also, biliary cancer and bladder cancer were elevated, but this was not statistically significant. Among women, breast cancer was elevated.

Significant study limitations: No industrial hygiene characterization of exposure. Exposure misclassification resulting from the use of "solvent authorization" rather than "solvent usage" as an exposure measure.

3) Ott et al. (1985) Dow Chemical employees at the Pittsburgh/Walnut Creek and Torrance facilities

Note: DCM was not separated from other solvents

Method: Retrospective cohort mortality study on males employed one or more years at a chemical manufacturing company between 1940 and 1969 with vital status ascertainment through 1979. Worker mortality was compared to U.S. white male population.

Exposure measures: Exposures to chlorinated methanes was indexed by employment exposure duration in years based upon job assignments. DCM was not separately identified.

Range of outcome variables considered: Twenty-nine ICD-based cause-of-death categories plus *all* causes of death.

Sample size: There were 1,919 white male workers and 390 deaths.

Control over extraneous variables: The sample was restricted to white males. Age at entry, interval since entry, and socioeconomic status at entry was controlled.

Cancer latency: Thirty-nine years maximum.

Significant findings: None. There were no statistically significant elevations for any specific causes of death.

Significant study limitations: DCM not separately identified.

The Ott et al. (1983a-1983e) studies were designed to examine cardiovascular effects, and consequently the study period was too short to allow for latency of site-specific cancers. An update of the Ott et al. (1985) study, based on longer follow-up, indicated possible elevation of liver and biliary tract cancers (TSCA section 8(e) submission no. 8eHQ-0198-0772 FLWP et seq. 1989).

Reviews in Literature

The epidemiological studies of occupational inhalation of DCM have been covered in two reviews of the health effects of solvents. One question addressed in the reviews is whether or not

the epidemiological studies provide human evidence consistent with that based on animal toxicology, i.e., liver cancer, lung cancer, and biliary tract cancer (Lynge et al. 1997). Another question addressed is whether or not predictions of increased cardiovascular disease based upon DCM metabolism to CO are seen in human epidemiology studies (Wilcosky and Simonsen 1991). The DCM epidemiology studies have been criticized for assumptions made in exposure estimation and interpretation of the results relative to extrapolations of the toxicological findings (Hearne 1991, Stayner et al. 1993, Tollefson 1988, 1991). Stayner and Bailer (1993) and Tollefson (1990) present the case that the epidemiological findings are consistent with the positive animal bioassay results, while Hearne (1991) presents the case that the human findings are inconsistent with the upper bound potency extrapolation from the mouse bioassay and show no DCM effect.

After several of the epidemiological studies (Cocco et al. 1994, 1999, Dosemeci et al. 1994, Gomez et al. 1994, Hearne et al. 1987, Lanes et al. 1990) were completed and findings presented which supported extrapolations from animal toxicology, the mortality follow-up was extended to try to address the veracity of the initial findings. Based upon the longer period of mortality ascertainment, the initial findings of excess mortality and interpretations based upon them were not supported (Hearne et al. 1990, Hearne and Pifer 1999, Lanes et al. 1993).

Several of the epidemiology studies have been heavily criticized based upon allegedly flawed exposure measures. The Heineman et al. (1994) finding of a relationship between DCM exposure and astrocytic brain cancer has been criticized by Norman (1996) for flawed exposure characterization. However, Dosemeci et al. (1994) indicated that the degree of exposure misclassification could be reduced by the introduction of these features into the job-exposure matrix. The study of Hearne et al. (1987) which suggested excess pancreatic cancer, and the study of Lanes at al. (1990) which reported biliary passage and liver cancer excesses, have been criticized by Hertz-Piccotto and Neutra (1994) on the grounds of the assumptions made in relation to dose and carcinogenic effect. However, the positive findings from Hearne (1987) and Lanes (1991) were in effect withdrawn (i.e., the findings failed to be supported) when the mortality follow-up periods were extended (Hearne et al. 1990, Lanes et al. 1993), indicating that the shorter mortality follow-up period was a likely study design limitation in each of the two studies.

Overall, the reviewers have stated that, in general, it is better to use the weight of evidence from epidemiological data to assess risk in humans than to extrapolate from animal toxicology. However, the reviewers have reported that the DCM epidemiological studies are not conclusive (Hearne and Lednar 1994). IARC (1999) in its recent updates of DCM human carcinogenicity data evaluation concluded “For no type of cancer was there a sufficiently consistent elevation of risk across studies to make a causal interpretation credible.” IARC (1999) therefore retained DCM as a Group 2B carcinogen.

Review by OEHHA Scientists

Based upon these occupational epidemiological studies of DCM inhalation exposure, and the reviews and analyses provided for them, there does not appear to be compelling evidence that low-level occupational DCM inhalation presents a human cancer risk. In these studies, cigarette smoking is a potential confounding factor. Cigarette smoking was judged to be similar in the Kodak cohort to the general population (Friedlander et al. 1978, Hearne et al. 1990), and was controlled in regression analyses in the Celanese cohort (Ott et al. 1985a-1985e). Workplace smoking was controlled in the Brantham cohort (Tomenson et al. 1997). Overall, it does not appear that cigarette smoking in the cohorts studied was likely to bias results, especially in a

positive direction. Animal bioassay studies, by contrast, indicated a risk for lung and liver neoplasms through both inhalation and drinking water ingestion (NTP 1986, Serota et al. 1986a, 1986b).

The earlier epidemiological finding of liver and biliary tract cancer related to DCM exposure were not supported with longer mortality ascertainment, indicating that study design rather than toxicologic effect was principally responsible for the findings. However, the epidemiological studies do not appear to be capable of ruling out adverse health effects either, due in part to their lack of control over potentially confounding factors, such as cigarette smoking, their lack of individual worker exposures, and their inability to separate DCM from other solvent exposures.

There are no published epidemiological studies of the health effects associated with ingestion of DCM from contaminated drinking water, although animal bioassays in mice and rats were positive for ingestion exposure (Serota et al. 1986a, 1986b). A basis upon which occupational inhalation effects can be extrapolated to consumption of contaminated drinking water has not been addressed in the literature.

Table 8. Summary of Statistically Significant Findings from Major Dichloromethane Inhalation Epidemiological Studies

Study and disease outcome	Significant findings and comments
A. The Eastman Kodak cohort	
1. Friedlander et al. (1987)	None
2. Hearne and Friedlander (1981)	None
3. Hearne et al. (1987)	None (Suggestive pancreatic cancer)
4. Hearne et al. (1990)	None (No suggestion of pancreatic cancer)
B. The Hoechst Celanese cohorts	
1. Ott et al. (1985)	None (Clinical differentials related to exposure)
2. Lanes et al. (1990)	Biliary passages/liver cancer (SMR 5.75 ranging from 1.82 to 13.78) Accidents (SMR 1.64 ranging from 1.05 to 2.47)
3. Lanes et al. (1993)	None
C. The Amcelle cohort	
1. Gibbs et al. (1996)	None (Two findings inconsistent with hypotheses)
D. The Brantham cohort	
1. Tomenson et al. (1997)	None

EXPOSURE ASSESSMENT

The DCM exposure assessment is mainly adopted from Bogen et al. (1992). Details of the methodology, especially on estimating uptake through the skin and by inhalation, have been discussed recently by International Life Sciences Institute (ILSI 1999). Three exposure routes, ingestion, inhalation through volatilization from showers and other indoor water uses, and dermal contact through bathing and washing, were used to estimate the total dose of DCM received by individuals in dwellings with contaminated tap water (Shehata 1985, Wallace 1987). The distribution of estimated exposure through these three routes using the Bogen et al. (1992)

calculations was 35 percent, 61 percent, and 4.7 percent, respectively. Calculations using CalTOX™ resulting in a distribution of 38 percent, 60 percent, and two percent, respectively, confirmed the estimates.

Background

Actual human exposure to DCM in water was difficult to assess for California residents because analytical data were not available for all water systems. However, DCM was found in a few locations in California groundwater as described earlier. Actual dose estimates used in this assessment were based on the statewide survey of organic chemical contaminants in groundwater of large and small water systems, as determined by analyses mandated by AB1803 (DHS 1986, 1990).

Studies of human exposure to volatile organic chemicals (VOCs) in drinking water suggest that humans are exposed to these chemicals by routes other than ingestion (McKone 1987). For example, Brown et al. (1984) estimated that dermal absorption of some VOC's could account for 29 to 91 percent of the total dose of these chemicals received through the use of contaminated tap water. Exposure through inhalation of VOCs volatized from tap water was suggested to contribute from approximately 50 percent to as much as six times that due to normal ingestion of water (Andelman 1985, Cothern et al. 1984, 1986; Forster and Chrostowski 1986, Prichard and Gesell 1981). Three exposure routes were used to estimate the total dose of DCM received by individuals in dwellings with contaminated tap water: 1) ingestion, 2) inhalation through volatilization from showers and other water uses, and 3) dermal contact through bathing and washing.

Assumptions

In order to calculate the daily dose of DCM received by an individual from contaminated tap water, it was necessary to estimate average daily intake of fluids, volumetric breathing rate, body weight, and body surface area, to name only a few parameters (Bogen et al. 1992). Physiological assumptions used in the exposure assessment for newborn up to two years as infants, two to 16 years as children, and over 16 years of age as adults are shown in Table 9 which are adopted from International Commission on Radiological Protection's Reference Man (ICRP 1975). The adult parameters used in this exposure assessment were the averages of males and females.

Table 10 lists values of the hourly-breathing rate by age and activity level. Also provided are the daily average breathing rates based on the time spent at rest or awake and the daily average breathing rate per unit body weight. These values represent the volume of air that enters and leaves the lungs within a one-hour period. Based on information in this table, a single breathing-rate-per-unit-body-weight for male and female adults of 14 L/kg-hour for the daily average air intake is used. However, to estimate time-varying exposures it is assumed that the infant, child, and adult, respectively, breathe 29, 24, and 17 L/kg-hour during waking hours and 11, 9, and 6.1 L/kg-hour while resting.

Table 11 lists reference values for intake of fluids by infants, children, and male and female adults. Other sources of fluids refer to all intakes of fluids, exclusive of milk and tap water consumption. It should be noted that intake of fluids in beverages such as coffee, tea, fruit juices, and soft drinks may be indirect tap-water consumption. Also listed in Table 11 is the fluid intake per unit body weight. For infants and children, these ratios are 0.11 and 0.044 L/kg-day, respectively. For adults, the ratio of intake varies from typical value of about 0.026 L/kg-day to

values as high as 0.05 L/kg-day at high environmental temperatures or for moderately active adults (ICRP 1975).

Table 9. Human Body weight and Surface Area by Age and Sex (from ICRP 1975)

Age (years)	Sex	Mass (kg)[a]	Surface Area [a] (m^2)
Newborn	Male/female	3.5 ± 0.6	0.22 ± 0.02
1	Male/female	10 ± 2	0.47 ± 0.07
2	Male/female	12 ± 2	0.54 ± 0.06
4	Male/female	18 ± 2	0.73 ± 0.06
8	Male/female	26 ± 5	0.96 ± 0.1
12	Male/female	41 ± 8	1.3 ± 0.2
16	Male	62 ± 8	1.7 ± 0.1
	Female	55 ± 8	1.6 ± 0.1
20	Male	70 ± 10	1.8 ± 0.1
	Female	58 ± 9	1.6 ± 0.1
40	Male	75 ± 10	1.8 ± 0.1
	Female	62 ± 10	1.7 ± 0.2
Infant newborn to 2	Male/female	8.5 ± 3	0.42 ± 0.1
Child 2 to 16	Male/female	32 ± 16	1.1 ± 0.4
Adult 16 to 70	Male	73 ± 10	1.8 ± 0.1
	Female	60 ± 9	1.6 ± 0.1

[a]Arithmetic mean ± one standard deviation

Note: surface area is calculated as:

$$\text{surface area (SA, in } m^2) = \frac{4 \times BW\,(kg) + 7}{BW\,(kg) + 90}$$

Table 10. Reference Breathing Rates for Infants, Children, and Adults (ICRP 1975)

Activity	Infant (1-year)	Child (10-year)	Adult female	Adult male
	L/hour (hour/day)	L/hour (hour/day)	L/hour (hour/day)	L/hour (hour/day)
Working, light activity, or recreation	250 (10)	780 (16)	1100 (16)	1200 (16)
Resting	93 (14)	288 (8)	360 (8)	450 (8)
Daily average (L/hour)	160	620	850	950
Daily average breathing rate per unit body weight (L/kg/hour)	19	19	14	13

Table 11. Fluid Intakes for Infants, Children, and Adults (from ICRP 1975)

Fluid (L/day)	Infant (1-year)	Child (10-year)	Adult female	Adult male
Milk	0.9	0.45	0.2	0.3
Tap water	--	0.2	0.1	0.15
Other [a]	--	0.75	1.1	1.5
Total fluids	0.9	1.4	1.4	2.0
Fluid intake per unit body weight (L/kg/day)	0.11	0.044	0.023	0.027

[a]Includes tea, coffee, soft drinks, beer, and other beverages.

Water-Based Pathways and Dose-Rate Factors

In this section "pathway-specific absorbed dose-rate" (PAD) factors are developed for water-based exposures. Three pathways are considered as follows: water ingestion, inhalation, and dermal absorption. For a given exposure pathway as i (where i = 1 for ingestion, i = 2 for

inhalation, and i = 3 for skin absorption), the lifetime TWA effective dose rate, E_i (in mg/kg-day) is calculated as follows:

$$E_i = F_i M_i C_w$$

where M_i is the corresponding metabolism factor converting absorbed to effective dose for the given pathway, F_i is the overall weighted sum of unit PAD factor, and C_w (mg/L) is the concentration of DCM in water.

For all age groups it is assumed that $M_1 = 0.906$ and $M_2 = M_3 = 0.602$, based on the PBPK model for DCM by Andersen et al. (1987a, 1987b). The overall exposure factor, F_i, as the weighted sum of the PAD factors, f_i (age group), for each of the three age categories is calculated as follows:

$$F_i = (2/70)\, f_i(\text{infant}) + (14/70)\, f_i(\text{child}) + (54/70)\, f_i(\text{adult})$$

The factors, 2/70, 14/70, and 54/70, reflect the fraction of time the population cohort spent in each of the age categories.

Water Ingestion

For the water-ingestion pathway (i = 1), the unit PAD factor for each age category is obtained by dividing daily water intake by body weight. The ratio of fluid intake to body weight for each age group comes from Table 11. An average of female and male adult fluid intake of 0.025 L/kg/day for an average adult from Table 11 was used to generate F_1. We also use data compiled by the ICRP (1975) on fluid intake by adults at high environmental temperatures and during moderate activity. The ICRP (1975) reports that at high environmental temperatures (to 32°C) average adults consume 2.8 to 3.4 L/day of fluids and that moderately active adults can consume 3.7 L/day. Using an average adult weight of 66.5 kg (mean of 73 kg for male and 60 kg for female from Table 9), this corresponds to a fluid intake of 0.0056 L/kg/day, which is the conservative assumption for fluids uptake. Thus the exposure factor F_1 is calculated as follows:

$$F_1 = (2/70 \times 0.11) + (14/70 \times 0.044) + (54/70 \times 0.025) \quad \text{(average)}$$

$$= 0.031 \text{ mg/kg-day per mg/L, and}$$

$$F_1 = (2/70 \times 0.11) + (14/70 \times 0.044) + (54/70 \times 0.056) \quad \text{(conservative)}$$

$$= 0.055 \text{ mg/kg-day per mg/L.}$$

The "typical" value of 0.031 mg/kg-day per mg/L is similar to the value of 0.028 mg/kg-day per mg/L obtained under the assumption that daily intake of drinking water over a lifetime approximates 2 L/day and body weight of 70 kg. The upper-bound limit corresponds to a lifetime average daily fluid intake of 3.8 L/day and body weight of 70 kg. These "typical" and upper bound values are within the range reported by Ershow and Cantor (1989) for age-, region- and season-average intakes of tap water in the Western U.S.

Inhalation Exposure

Several researchers have addressed the relative contribution of the respiratory pathway to overall human exposures from VOCs in tap water. All have found that volatile compounds in water supplies can result in inhalation exposures that are comparable to the ingestion pathway. Prichard and Gesell (1981) found that public water supplies provide a major pathway for indoor exposures to radon and measured the amount of radon transferred to air from water during various household activities. Cothern et al. (1984, 1986) found that the respiratory uptake of

VOCs from household air attributable to tap water is approximately equal to oral uptake from fluids. Andelman (1985) developed a model shower that he used to study human exposures while showering or bathing. He found that for a volatile pollutant, overall indoor inhalation exposure might be as much as six times higher than direct ingestion exposures. Forster and Chrostowski (1986) developed an integrated household exposure model for assessing human uptake of VOCs from tap water.

McKone (1987, 1989) developed a model that describes the daily concentration profile of VOCs within a dwelling. The model divides the indoor air volume into three compartments - the shower/bath stall, the bathroom, and the household volume. This model is used to calculate two bounding PAD factors that correspond to water use in the indoor environment. One corresponds to the average lifetime daily exposure while the other represents a more conservative estimate of exposure in which the model parameters are set at values that provide the upper limit on exposure estimates. For the typical home, it is assumed that the household has four occupants and uses 900 L/day of water containing a very low concentration of DCM and that DCM has low-concentration water-to-air transfer properties similar to radon-222 (Cothern et al. 1984, 1986).

The time-dependent concentration profile of DCM in shower stall, bathroom, and household air and the resulting average lifetime absorbed doses were estimated using a modification of the McKone (1987, 1989) model. Assumptions made are:

For typical exposures:

- Occupants spend 100 percent of their time in the house from 11:00 p.m. to 7:00 a.m.
- Bathroom is used for showers/baths from 7:00 a.m. to 8:00 a.m.
- Each adult and child spends 20 minutes in the bathroom during the period from 7:00 a.m. to 9:00 a.m.
- Each adult spends 10 minutes in the shower or bath.
- Adults spend 25 percent of the time from 7:00 a.m. to 11:00 p.m. in the house.
- Children spend an average of 20 minutes/week in showers or baths.
- Children spend 60 percent of the time between 7:00 a.m. to 11:00 p.m. in the house.
- Infants spend 100 percent of their time in the house and two percent of that time in a bathroom.

For upper-bound doses:

- Each adult and child spends 40 minutes in the bathroom between 7:00 a.m. and 9:00 a.m.
- Each adult spends 20 minutes in the shower or bath.
- All age groups spend 100 percent of their time in the house.
- Children spend an average of 40 minutes/week in showers or baths.

Respired DCM was assumed to be totally absorbed in the alveoli at a resting rate of five L/kg/hour (348 L/hour) for a reference 70-kg adult (Andersen et al. 1987a, 1987b, U.S. EPA 1988). The daily average alveolar ventilation rate for all age groups, including 16 hours of assumed light activity per day, was taken to be 10.9 L/kg/hour for a reference 70-kg male adult. The resulting PAD factors are:

F_2 = 0.048 mg/kg-day per mg/L (typical households), and

F_2 = 0.097 mg/kg-day per mg/L (upper-bound estimate).

These values are based on the assumption that an adult showers everyday and that children bathe every second day. Table 12 summarizes the relative contribution to the PAD factor, F_2, from each age category and household compartment. For adults or children who take baths instead of showers these numbers are likely to be reduced somewhat. We have not examined the extent of reduction that taking baths in place of showers would give. The summary in Table 12 reveals that exposures to adults in the shower and bathroom are the major contributors to indoor inhalation exposures attributable to contaminated water. A local sensitivity analysis of the different parameters involved in the calculation of the PAD factor for the inhalation route has been prepared by McKone (1987). According to that analysis, this factor was most sensitive to increases in the following parameters: the uptake fraction in the lung, the transfer efficiency of a VOC in shower water to air, the water use of individuals in showers, and the ratio of breathing rate to adult body weight.

Although the assumption that adults spend 25 percent of the time from 7:00 a.m. to 11:00 p.m. in the house may seem low, this is a plausible value for a typical adult who spends 10 hours per day in work and travel, eight hours in sleep, leaving six hours of leisure time of which we assume roughly two-thirds is actually spent in the home. Furthermore, it is assumed that roughly three-quarters of all adults work outside the home and that those who do not can be accounted for by the upper-bound estimate.

Table 12. Percent Contribution to the Lifetime Time-Weighted-Average (TWA) Pathway-Specific-Absorbed-Dose-Rates (PAD) Factor F_2 (inhalation) from Specific Age and Household Exposures

Exposure		Percent Contribution	
Typical household PAD factor		**Typical PAD factor**	**Upper-bound PAD factor**
Adult	Shower	51	42
	Bathroom	20	25
	Remainder of house	7.9	14
Child	Shower	5.2	4.2
	Bathroom	7.2	9.0
	Remainder of house	7.0	4.9
Infant	Bathroom	0.17	0.16
	Remainder of house	1.2	0.56
TOTAL		100 (99.67)	100 (99.82)

Dermal Absorption

Over the last 20 years, several investigators have examined the transport of dissolved chemicals through skin (Bronaugh 1985, Brown et al. 1984, Cothern et al. 1984, 1986; Forster and Chrostowski 1986, Riihimaki and Pfäffli 1978, Scheuplein and Blank 1971, Stewart and Dodd 1964). Although a complex process, dermal uptake of compounds occurs mainly by passive diffusion through the stratum corneum.

In order to develop a PAD factor for dermal absorption, it is assumed that dermal exposure occurs during bathing and showering. To determine the PAD factor for dermal absorption, the following assumptions were made:

- Resistance to diffusive flux through layers other than the stratum corneum is negligible.
- Steady-state diffusive flux is proportional to the concentration difference between the skin surface.
- An adult spends from 10 to 20 minutes in a bath or shower each day.
- During bathing, roughly 80 percent of the skin is in contact with water, and during showers, roughly 40 percent of the skin is in contact with water.
- Children and infants spend approximately one hour per week in bathing or swimming (NRC 1977).

The absorbed dose rate factor, F_3 in mg/kg-day per mg/L from dermal contact is given by the expression

$F_3 = J_s \times \tau \times f_s \times SA$

where

J_s = steady-state flux across the stratum corneum (mg/cm^2/hour),

τ = duration in the shower or bath (hours),

f_s = fraction of the skin surface in contact with water (unitless), and

SA = surface area of the skin (cm^2).

It is assumed that chemical transport across the skin follows Fick's law, so that the flux J_s across skin tissue is given by

$J_s = K_p \Delta C_s$

where

Kp = effective permeability constant across the stratum corneum for the duration of the shower/bath (L/cm^2/hour), and

ΔC_s = concentration difference of the solute across the tissue (mg/L).

Based on experiments with hairless guinea pigs, Bogen et al. (1992) have determined that the effective K_p for aqueous chloroform applied to human skin is likely to be between 0.0001 and 0.0002 L/cm^2/hour. The upper value of this range is used in the present analysis to estimate an upper bound on potential dermal absorption of DCM in humans. The Bogen et al. (1992) study was among those from which McKone and Howd (1992) obtained experimental data on skin permeability for a variety of chemicals with which they developed a general model capable of predicting percutaneous absorption of these compounds. The McKone and Howd (1992) model predicts that for a compound such as DCM during a short exposure period (< 15 minutes), the effective permeability is given by the following equation:

$$\text{Kp (effective in L/cm}^2\text{-hour)} \leq \frac{\delta_{skin}[(6.4 \times 10^{-3}) + (2.5 \times 10^{-3}) \times K_{ow} \times 0.8]}{2}$$

where δ_{skin} is the stratum corneum thickness (~ 2.7×10^{-3} cm for hydrated skin) and K_{ow} is the octanol-water partition coefficient (93 for DCM) (McKone and Howd 1992). Making these substitutions give $K_p \leq 4.2 \times 10^{-4}$, in accordance with the assumption that the K_p value of

2.0×10^{-3} for chloroform based on the data of Bogen et al. (1992) is likely to overestimate dermal absorption of DCM in humans.

For dilute solutions, ΔC_s is approximately equal to the chemical concentration at the skin surface, C^s_w. However, the concentration at the skin surface is not necessarily the same as the concentration in the tap water supply. For showers, it is assumed that $C^s_w = C_w$, the DCM concentration in tap water. But for bathing, in which water stands for a period of time, we use $C^s_w = B_w$ where B_w is the average water concentration over the period of the bath. Assuming an exponential loss of one-half of the dissolved DCM over a period of 10 minutes, $B_w = 0.72\ C_w$ during a 10-minute bath and $B_w = 0.54\ C_w$ during a 20-minute bath (Forster and Chrostowski 1986).

Making substitutions, the lifetime equivalent PAD factor for dermal absorption,

$$F_3 = K_p\ [2/70(\tau f_s SA/BW\ C^s_w)_{infant} + 14/70(\tau f_s SA/BW\ C^s_w)_{child} + 54/70(\tau f_s SA/BW C^s_w)_{adult}]$$

in which BW represents body weight in kg, SA represents surface area in cm^2, and τ the duration of bath or shower. Based on data from Table 9, the ratios SA/BW for adults, children, and infants are respectively 260, 340, and 490 cm^2/kg.

Thus, best estimate (assuming 10-minute bath for adults):

$$\begin{aligned} F_3 &= 0.0002\ L/cm^2/hour[(0.17\ hours/day \times 0.80 \times 490\ cm^2/kg \times 0.72\ C_w) + \\ &\quad 14/70(0.17\ hours/day \times 0.80 \times 340\ cm^2/kg \times 0.72\ C_w) + \\ &\quad 54/70(0.17\ hours/day \times 0.80 \times 260\ cm^2/kg \times 0.72\ C_w)] \\ &= 0.0055\ mg/kg\text{-}day\ per\ mg/L \end{aligned}$$

Higher estimate (assuming 20-minute bath for adults):

$$\begin{aligned} F_3 &= 0.0002\ L/cm^2/hour[2/70(0.17\ hours/day \times 0.80 \times 490\ cm^2/kg \times 0.72\ C_w) + \\ &\quad 54/70(0.17\ hour/day \times 0.80 \times 260\ cm^2/kg \times 0.72\ C_w)] + \\ &\quad 54/70(0.33\ hour/day \times 0.80 \times 260\ cm^2/kg \times 0.54\ C_w)] \\ &= 0.0073\ mg/kg\text{-}day\ per\ mg/L \end{aligned}$$

Summary of PAD Factors and Corresponding Effective Doses

Table 13 is a summary of the three PAD factors. The effective doses of DCM from groundwater that correspond to the PAD factors assuming a one-mg/L concentration of DCM in groundwater used for domestic consumption are given in Table 14. The results indicate that inhalation is expected to be the primary exposure route leading to biologically effective doses of DCM from water-based household sources and the dermal route is a minor contributor to the total biologically effective dose from tap water.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

The most sensitive noncarcinogenic effect by oral route is liver toxicity based on the Serota et al. (1986a) 24-month drinking water ingestion study with NOAELs of 5.85 and 6.47 mg/kg-day for male and female rats, respectively. A RfD of 0.06 mg/kg-day is then derived in IRIS (U.S. EPA 1999) with uncertainty factors of 100. A proposed Drinking Water Equivalent Level (DWEL) of 2 mg/L using 70 kg body weight and 2 L/day DWC can then be derived. However, in an earlier U.S. EPA (1992a) document based on the same studies, a rounded-down value of 0.05 mg/kg-day

was reported as the RfD to derive a proposed DWEL of 1.75 mg/L which was then rounded up to 2 mg/L using 70 kg body weight and 2 L/day DWC. Based on these values and 20 percent RSC, a lifetime HA of 400 ppb (or 0.4 mg/L) can be derived for DCM in drinking water based on noncarcinogenic effects. Other states also used this toxicity endpoint to develop regulatory guidelines for DCM as described later in this document.

Table 13. Summary of the Pathway-Specific-Absorbed-Dose-Rates (PAD) Factors for Dichloromethane

Pathway	**Fluid ingestion**	**Indoor inhalation**	**Dermal absorption**	**Total†**
Variable	**F_1**	**F_2**	**F_3**	**$F = SF_i$**
Best estimates:				
mg/kg-day per mg/L	0.031	0.048	0.0055	0.104
Equivalent Lifetime daily Fluid intake by 70-kg adult, L	2.2	3.4	0.38	6.0
Percent of total	37%	57%	6.5%	100%
Upper bounds:				
mg/kg-day per mg/L	0.055	0.097	0.0073	0.160
Equivalent Lifetime daily Fluid intake by 70-kg adult, L	3.8	6.8	0.51	11.1
Percent of total	35%	61%	4.7%	100%

†Columns two to four may not total to column five due to rounding.

Table 14. Summary of Effective Dichloromethane Doses Corresponding to Pathway-Specific-Absorbed-Dose-Rates (PAD) Factors Listed, Assuming 1 mg/L of Dichloromethane in Household Water

Pathway	Fluid ingestion	Indoor inhalation	Dermal absorption	Total†
Variable	**E_1**	**E_2**	**E_3**	**$E = SE_I$**
Best estimates:				
Lifetime TWA Effective dose (mg/kg-day)	0.028	0.029	0.0033	0.06
Percent of total	46%	48%	5.5%	100%
Upper bounds:				
Lifetime TWA Effective dose (mg/kg-day)	0.050	0.058	0.0044	0.112
Percent of total	44%	52%	3.9%	100%

†Columns two to four may not total to column five due to rounding.
TWA: time-weighted average

Carcinogenic Effects

Physiologically Based Pharmacokinetic Models

PBPK models are based on data from many sources; they attempt to simulate actual body organs or tissue groups (e.g., liver, muscle, fat), and are essentially predictive. These features are in contrast to classical or descriptive pharmacokinetic modeling that uses rather abstract compartments and interrelations to fit to specific data sets, usually for deriving pharmacokinetic constants and parameters. PBPK modeling has been applied in many chemical risk assessments to correct for metabolic saturation of high bioassay doses, to derive improved internal dose metrics, and to improve inter-route and inter-species extrapolations.

As described earlier in this report and in the OSHA (1997) Final Rule (see on the web at www.osha.slc.gov/sltc/methylenechloride/index.html), several PBPK models have been developed for DCM (Bogen et al. 1992). The PBPK model of Andersen et al. (1987a), Clewell et al. (1994), and Gargas et al. (1987a, 1991) was used by U.S. EPA as a basis for revising their DCM carcinogen risk assessment (U.S. EPA 1987b). Additional improvements in the PBPK modeling of DCM have focused on uncertainty analysis of key model parameters for rodent and human models (Clewell 1993, Clewell et al. 1994), dose metrics for cancer risk assessment in lung and liver target tissues (Andersen and Krishnan 1994, Andersen 1995), and the mechanism of DCM oxidation in mice with deuterated forms of DCM (Andersen et al. 1994).

Several authors (e.g., Clewell 1995, Andersen 1995) have discussed the question of the most appropriate dose metric to use in a DCM risk assessment. Typical metrics for carcinogenic chemicals are based on the area under the blood concentration times time curve for the parent compound or carcinogenic metabolite (AUC blood), the AUCs for the target tissues if the data or PBPK model allow, or the amount of parent chemical metabolized by the pathway yielding the

genotoxic and carcinogenic metabolite on a whole animal or target tissue basis. For DCM the metric suggested most often was the amount of GST conjugate metabolites formed per day per unit volume of target tissue (Andersen 1995). The PBPK model used by OSHA (1997) would allow determination of the latter metric for lung and liver target tissues with inhalation exposures. Any metric chosen must have biological relevance. For the dose response analysis below we have chosen to use a metric based on the amount of GST conjugates formed per day per liver (or lungs) from the PBPK model expressed on a body weight basis, i.e., mg per kg body weight per day rather than mg per L tissue per day. Both of these related metrics involve the assumption that the carcinogenic effects of the tissue doses are confined to the tissues in which they are generated. Also while the PBPK doses have the same units as an applied dose it should be remembered that they are target tissue specific metabolized doses derived by modeling.

Internal Dose Estimation

The carcinogenic mode of action of DCM, while not clearly established, appears most likely to be related to the generation of genotoxic metabolites via the cytosolic GST pathway. The GST pathway metabolizes DCM to formaldehyde and chloride ions via a postulated S-chloromethylglutathionate conjugate. Both formaldehyde and the S-chloromethylglutathionate are known to react with protein, RNA, and DNA. The PBPK model used here is essentially the same used by OSHA (1997). This approach is based on the PBPK model of Clewell (1993, 1995) as modified by Bois and Smith (1995), and Hattis (1995). It employs the following compartments: GI Tract, Fat, Liver, Poorly Perfused Tissues, Well Perfused Tissues, Lung, and Bone Marrow. In our hands the model was modified slightly to accommodate oral intake via the Liver compartment as well as inhalation exposure. The model was formulated in Stella II for Windows software (v. 3.0.7, High Performance Systems Inc., Hanover, New Hampshire 03755). The model units for the whole animal are moles, L, hour, moles/L and ppm in alveolar air. Simulations of up to 24 hours were run at 1,000 steps per simulated hour using the Runge-Kutta Four computation method.

The model parameters were obtained from the OSHA (1997) Final Rule, converted into model units and are listed in Table 19. Two sets of mouse parameters were used in the modeling: 1) the geometric mean values from the prior distributions used in the Bayesian analysis from OSHA (1997) Table VI-5; and 2) the median values of the posterior distributions from OSHA (1997) Table VI-9. OSHA (1997) used both non-independent Monte Carlo simulation and Bayesian analysis with independently collected laboratory data to improve estimates of the PBPK model parameters (posterior values). Despite this analysis some uncertainties still remain, for example, with respect to metabolic parameters and effects of polymorphisms (see discussion below).

Model simulations were run for 12 or 24 hours following inhalation or oral administrations of DCM to a mouse with a default body weight of 0.025 kg. For the oral doses 60, 125, 185, and 250 mg/kg-day from the Serota et al. (1986b) drinking water study were used. Despite the relatively low doses and weak response in this study there did appear to be a linear dose response. For the inhalation doses 2,040 and 4,052 ppm for six hours per day from the NTP (1986) study were used. The target tissues of liver from the oral study and liver and lung from the inhalation study were evaluated to determine the amount of GST pathway metabolites generated by each tissue per day. In all cases the relations between PBPK tissue doses and applied doses were linear. The PBPK tissue doses of moles per tissue per day were then expressed on a per kg body weight, adjusted to continuous daily basis (inhalation), and are referred to as the PBPK continuous doses (mg/kg-day) (Table 15). The applied and PBPK doses

for both sets of model parameters are given in Table 18. The linear regressions relating applied doses to continuous

PBPK doses are listed in Table 20. The PBPK doses from the posterior analysis (median) were lower for the oral doses and higher for the inhalation exposures than the prior (mean) analysis. In terms of goodness of fit to the quantal tumor incidence data there appears to be little difference between the two sets of model parameters. All the data sets were fit by the polynomial equation with acceptable χ^2 goodness of fit statistics ($p > 0.05$).

Cancer Slope Factors

Carcinogenic potency values for each set of parameters were determined using Tox_Risk (version. 3.5) software and are given in Tables 16 and 17. As shown in Table 16, for the prior parameter-based analysis the oral CSF value of 0.011 $(mg/kg\text{-}day)^{-1}$ was obtained from the Serota et al. (1986b) drinking water study for adenomas and carcinomas in male mouse liver. For the inhalation route, also from the prior parameters, the geometric mean of the CSF from combined liver adenomas and carcinomas of both male [0.1 $(mg/kg\text{-}day)^{-1}$] and female [0.125 $(mg/kg\text{-}day)^{-1}$] was 0.11 $(mg/kg\text{-}day)^{-1}$. With the exception of lung adenomas in female mice, all the lung based CSF values were lower than the liver values. The geometric mean of the male mouse lung carcinomas and the female mouse lung carcinomas was 0.02 $(mg/kg\text{-}day)^{-1}$. A combined potency for both lung and liver sites would be 0.13 $(mg/kg\text{-}day)^{-1}$. In general the CSFs derived from PBPK using the posterior model parameters were similar to the prior parameter-based values.

As shown in Table 17, the CSF for the combined liver adenoma and carcinoma of the oral route was 0.014 $(mg/kg\text{-}day)^{-1}$. For the inhalation route, also from the posterior parameters, the geometric mean of the CSFs from combined liver adenomas and carcinomas of both male [0.09 $(mg/kg\text{-}day)^{-1}$] and female [0.16 $(mg/kg\text{-}day)^{-1}$] was 0.12 $(mg/kg\text{-}day)^{-1}$. As above, the lung based CSFs were lower. The geometric mean of the male and female mouse lung carcinomas was 0.029 $(mg/kg\text{-}day)^{-1}$. A combined CSF potency estimate for both sites would be 0.15 $(mg/kg\text{-}day)^{-1}$.

The oral CSFs are similar to the previous oral potency value of 0.014 $(mg/kg\text{-}day)^{-1}$ calculated by OEHHA but the inhalation CSFs are higher than the previous OEHHA inhalation CSF value of 3.5×10^{-3} $(mg/kg\text{-}day)^{-1}$ (DHS 1989). The regressions in Table 20 can be used to calculate model-based inhalation CSFs in continuous air units or in calculating CSFs in external DCM equivalents. DCM equivalent CSF values for oral and inhalation prior parameters are 0.0099 $(mg/kg\text{-}day)^{-1}$ and 0.069 $(mg/kg\text{-}day)^{-1}$, respectively. For the posterior parameters the DCM equivalent CSFs are 0.004 $(mg/kg\text{-}day)^{-1}$ and 0.023 $(mg/kg\text{-}day)^{-1}$, respectively (see Table 21).

DCM was used as an example in evaluating reproducibility of carcinogenic potencies estimated in different rodent species (Bucchi et al. 1990). The potency values based on the PBPK tissue doses in mouse liver are considered the best dose metrics for assessing the cancer risk of DCM in humans. Although the posterior parameter-based analysis predicted negligible exposure to GST metabolites of DCM by the oral route, the animal data show a dose-response trend through this dose range and doses are predicted by the prior parameters. For the purpose of this risk assessment, the low dose linear dose response approach still appears to be justified for both oral and inhalation exposures to DCM.

Human PBPK Model

The human PBPK model described in the OSHA (1997) rule was also evaluated in short term 24-hour simulations. The simulations involved a single oral dose of 10 μg DCM (5 μg/L × 2 L/day) and an eight-hour inhalation exposure equivalent to an uptake of 20 μg DCM

(4 Leq/day). With the prior parameters, GST metabolites produced in liver and lung were 2.63×10^{-4} and 5.96×10^{-5} mg/kg-day, respectively. From the mouse studies estimates of liver LED_{10} values (Table 18) were 9.6, 1.0, and 0.8 mg/kg-day; estimates of lung LED_{10} values were 3.2 and 2.1 mg/kg-day. The unscaled liver values obtained by multiplying by $(70/0.025)^{1/4}$ were 69.8, 7.3, and 5.8 mg/kg-day; and the lung values were 23.3, and 15.3 mg/kg-day. The geometric mean values of liver were 14.4 mg/kg-day; and of lung were 18.9 mg/kg-day. The unscaled mean CSFs would be 6.9×10^{-3} and 5.3×10^{-3} $(mg/kg\text{-}day)^{-1}$, for liver and lung, respectively. The calculated risks are 1.8×10^{-6} and 3.2×10^{-7}, from liver and lung, respectively. The combined risk for liver and lung cancer from a daily exposure to five ppb DCM in water is 2.1×10^{-6}. Thus the negligible risk level is 5/2.1 = 2.3 ppb. A similar analysis using the median posterior PBPK parameters gave a combined risk of 3×10^{-6} and a negligible risk level of 1.7 ppb. Since the human PBPK simulations were only 24 hours it is likely that the GST metabolites per day would be somewhat higher if the simulations were conducted for a longer period and a steady state achieved. Thus the risk estimates may be somewhat underestimated. Also if human targets are more sensitive than mice to DCM induced cancer, the risk will be further underestimated. Negligible risk values based on animal data with interspecies scaling range from equivalent to 10-fold lower than these estimates and the mouse to human scaling factor is 7.3-fold.

This analysis assumes that target tissue doses/kg body weight will give equivalent effects in mice and in humans. The human PBPK model is based largely on *in vitro* data from a limited number of human subjects. There are significant uncertainties concerning all of the human metabolic parameters (i.e., V_{max} and K_m for the MFO pathway, K_{FC} for the GST pathway, and A1 and A2, the lung/liver fractional rates for MFO and GST pathways, respectively). More data are needed to adequately understand the extent of human polymorphisms including early life and in utero pharmacokinetics. In addition to such pharmacokinetic questions the area of DCM pharmacodynamics presents even greater uncertainties. While advances in studying the mouse lung and liver tumors may improve risk assessment for mice (e.g., dose modeling), the relevant endpoint in humans and appropriate pharmacodynamic model are unknown. The only fact at present is that humans possess (at least a fraction of the population) the relevant enzymes that are thought to be involved in the key toxifying/detoxifying DCM pathways. There are no significant tumor results in humans. While a working hypothesis could couple PBPK target tissue (mouse) concentrations to effects caused by formaldehyde-induced DNA cross links or possible S-chloromethylglutathione derived adducts in DNA or related macromolecules, and equivalent concentrations could be predicted for humans based on human PBPK, the nature and extent of the equivalent human response is unknown as is the appropriate dose metric for possible DCM-induced human tumors. Thus in order to estimate a safe drinking water level for humans the animal cancer data and derived risk estimates should be used instead of the human PBPK model.

Assessment of Human Cancer Risk

The objective of this document is to develop a health protective concentration for DCM in drinking water assuming possible lifetime exposures by the oral, dermal and inhalation routes resulting from the normal household uses of DCM contaminated tap water. The analysis presented above addressing cancer risk is based largely on the OSHA (1997) DCM assessment for occupational inhalation exposures and the mouse cancer studies of Serota et al. (1986a, 1986b) and NTP (1986). In developing this assessment of human cancer risk we have evaluated relevant animal cancer data from the two principal routes of anticipated human exposure (oral via drinking water and inhalation) and we used a PBPK model which we modified to incorporate oral exposure. Unlike the OSHA assessment we used two sets of central estimates of PBPK

model parameters rather than upper bound estimates. The PBPK model predicted target tissue doses of

the presumptive carcinogenic agent(s), metabolites generated by the GST pathway, at DCM doses, which resulted in cancers in the animal studies. A polynomial dose-response equation was fit to the tissue metabolite doses (mg GST metabolites/L tissue-day × L tissue/kg body weight = mg/kg-day) and the respective quantal tumor data. Two methods of low dose extrapolation were used to calculate carcinogenic potencies or slope factors, the linearized multistage model (LMS) and the LED_{10} linear method. We used ¾ power body weight scaling to obtain human equivalents from the animal data. Based on the oral and inhalation slope factors for each parameter set we derived additional slope factor values for inhalation unit risks and DCM equivalents based on regressions of tissue GST metabolite concentrations against applied DCM doses. The latter included external DCM equivalents for oral exposure and internal DCM equivalents for 70 kg humans inhaling 20 m^3/day, assuming either complete or 75 percent absorption based on the evaluation of Bogen et al. (1992) (Table 21).

Another approach we employed was to evaluate a human PBPK model, with the OSHA (1997) parameters, in a 24-hour simulation of maximum oral and inhalation DCM exposure likely to occur under current state and federal drinking water standards of 5 ppb. The model predicted tissue metabolite concentrations, which were compared with levels causing cancer in the animal studies assuming a human response equal to that of the mouse for a given GST metabolite concentration. Due to the greater uncertainties of pharmacokinetics, including metabolic parameters and polymorphisms, of the human PBPK model and risk estimates based on it we decided not to rely on its predictions, although they were similar to some of the lower animal-based risk estimates. Also there is considerable uncertainty about the human pharmacodynamics of DCM not simply the extent of tumor response in humans versus rodents but also tumor site concordance. The use of the human PBPK model assumes equal sensitivity and site concordance.

Uncertainties also exist to a lesser extent, with the animal PBPK model parameters and as noted above we chose to use central estimates in the PBPK model and 95 percent lower bounds in the dose-response assessment (LED_{10}) rather than OSHA's approach of the 95 percent upper bound on the metabolites and the maximum likelihood estimate of risk. Overall we believe that the animal-based cancer slope factors scaled to human equivalent represent the best basis for human cancer risk assessment of DCM in drinking water at present as they make no unjustified assumptions about potential DCM-induced tumors in humans. Among the various animal-based values those based on the pharmacokinetic tissue dose (mg GST metabolites/kg-day) are preferred since they are closest to the site of carcinogenic action and involve the fewest adjustments or other manipulations.

Table 15. Target Tissue Doses in Mice Exposed to Dichloromethane Estimated by Physiologically Based Pharmacokinetic (PBPK) Model

Study, dose route	Tumor site, type, parameter set	Applied dose	GST metabolites (mole/day) [1]†	GST metabolites (mg/L/day) [2]†	Continuous (mg/kg-day) [3]†
Serota et al. 1986b, drinking water ad lib	Liver, adenoma and carcinoma, Prior	0 mg/kg-day	0	0	0
		60	8.4×10^{-6}	632	28.4
		125	1.9×10^{-5}	1,427	64.1
		185	2.9×10^{-5}	2,212	99.4
		250	4.6×10^{-5}	3,450	155.1
	Posterior	0 mg/kg-day	0	0	0
		60	5.7×10^{-24}	-	-
		125	3.7×10^{-6}	252.8	12.6
		185	1.2×10^{-5}	781.4	38.9
		250	2.6×10^{-5}	1,787	89.1
NTP 1986, inhalation, 6 hour/day, 5 day/week	Liver , adenoma and carcinoma, Prior	0 ppm	0	0	0
		2040	3.7×10^{-5}	498.8	22.4
		4,052	8.4×10^{-5}	1,138	50.8
	Posterior	0 ppm	0	0	0
		2,040	3.6×10^{-5}	436.8	21.8
		4,052	1.0×10^{-4}	1,238	61.9
	Lung, adenoma and carcinoma, Prior	0 ppm	0	0	0
		2,040	1.0×10^{-4}	7,962	63.7
		4,052	2.1×10^{-4}	16,151	129.2
	Posterior	0 ppm	0	0	0
		2,040	1.0×10^{-4}	8,493	84.9
		4,052	2.8×10^{-4}	17,047	170.4

†Note:

[1] The cumulative metabolism via the GST pathway in the target tissue in moles per day from the PBPK model 24-hour simulation.

[2] mg GST metabolites (as DCM)/L tissue/day:
drinking water = (mole/day $\times$ 84.93 g/mole $\times$ 1000 mg/g)/(1.125×10^{-3} L/liver),
inhalation = (mole/day $\times$ 5/7day $\times$ 6/24 hour $\times$ 84.93 g/mole $\times$ 1000 mg/g)/tissue volume L.

[3] mg GST metabolites/kg body weight/day:
drinking water = (mole/day $\times$ 84.93 g/mole $\times$ 1000 mg/g)/(0.025 kg/animal),
inhalation = (mole/day $\times$ 5/7day $\times$ 6/24 hour $\times$ 84.93 g/mole $\times$ 1000 mg/g)/(0.025 kg/animal).

Table 16. Potency Estimates for Dichloromethane Based on the Occupational Safety and Health Administration (OSHA) Physiologically Based Pharmacokinetic (PBPK) Prior Mean Parameters (as in Table VI-5 in OSHA 1997 Final Rule, p. 1546)†

Study	Sex, species	Site, type	Route	q_1* (mg/kg-day)$^{-1}$	CSF (mg/kg-day)$^{-1}$
Serota et al. 1986b	Male mouse	Liver adenomas + carcinomas	Water ad lib	0.012	0.010
NTP 1986	Male mouse	Liver adenomas + carcinomas	Inhalation	0.113	0.10
	Female mouse	Liver adenomas + carcinomas	Inhalation	0.13	0.125
	Male mouse	Lung adenomas	Inhalation	0.051	0.045
	Male mouse	Lung carcinomas	Inhalation	0.033	0.031
	Female mouse	Lung adenomas	Inhalation	0.071	0.0625
	Female mouse	Lung carcinomas	Inhalation	0.054	0.0148

†Note:
Carcinogenic potencies are human q_1* values determined by the linearized multistage model (Tox_Risk v. 3.5) and carcinogen slope factors defined as $0.1/LED_{10}$ where LED_{10} is the 95 percent lower bound on the dose giving a 10 percent tumor incidence. Both estimates were adjusted for human equivalence with (body weight)$^{3/4}$ scaling.

Table 17. Potency Estimates for Dichloromethane Based on the Occupational Safety and Health Administration (OSHA) Physiologically Based Pharmacokinetic (PBPK) Posterior Median Parameters (as in Table VI-9 in OSHA 1997 Final Rule p. 1551)†

Study	Sex, species	Site, type	Route	q_1* (mg/kg-day)$^{-1}$	CSF (mg/kg-day)$^{-1}$
Serota et al. 1986b	Male mouse	Liver adenomas + carcinomas	Water ad lib	0.016	0.014
NTP 1986	Male mouse	Liver adenomas + carcinomas	Inhalation	0.10	0.090
	Female mouse	Liver adenomas + carcinomas	Inhalation	0.17	0.16
	Male mouse	Lung adenomas	Inhalation	0.038	0.029
	Male mouse	Lung carcinomas	Inhalation	0.024	0.024
	Female mouse	Lung adenomas	Inhalation	0.054	0.048
	Female mouse	Lung carcinomas	Inhalation	0.040	0.036

†Note:
Carcinogenic potencies are q_1* values determined by the linearized multistage (LMS) model (Tox_Risk version 3.5) and cancer slope factors (CSFs) defined as $0.1/LED_{10}$ where LED_{10} is the 95 percent lower bound on the dose giving a 10 percent tumor incidence. Both estimates were adjusted to human equivalence with (body weight)$^{3/4}$ scaling.

Table 18. Dosimetrics and Fit Statistics for Prior and Posterior PBPK Analyses†

Study	Sex of mouse, site, type, quantal responses	Applied Doses	PBPK doses (mg/kg-day) continuous lifetime	Goodness of Fit Statistics C^2, p, k	ED_{10}/LED_{10} (mg/kg-day)
Serota et al. 1986b	Male, Liver A + C 24/125, 51/200, 30/100, 31/99, 35/125	0, 60, 125, 185, 250 mg/kg-day	0, 28.4, 64.2, 99.4, 155.1 **0, 0, 12.6, 38.9, 89.1**	3.67, 0.30, 4 **5.77, 0.12, 4**	18.0/9.6 **17.2/7.3**
NTP 1986	Male, Liver A + C 22/50, 24/49, 33/49	0, 2,040, 4,052 ppm, 6 hour/day, 5 day/week	0, 22.4, 50.8 **0, 21.8, 61.9**	0.0047, 0.95, 2 **0, 1, 2**	3.4/1.0 **3.6/1.1**
	Female, Liver A + C 3/50, 16/48, 40/48	0, 2,040, 4,052 ppm, 6 hour/day, 5 day/week	0, 22.4, 50.8 **0, 21.8, 61.9**	0, 1, 2 **0, 1, 2**	1.8/0.8 **1.3/0.6**
	Male, Lung A 3/50, 19/50, 24/50	0, 2,040, 4,052 ppm, 6 hour/day, 5 day/week	0, 63.7, 129.2 **0, 84.9, 170.4**	0.98, 0.32, 2 **0.92, 0.34, 2**	3.0/2.2 **3.8/3.5**
	Male, Lung C 2/50, 10/50, 28/50	0, 2,040, 4,052 ppm, 6 hour/day, 5 day/week	0. 63.7, 129.2 **0, 84.9, 170.4**	0.0074, 0.93, 2 **0.02, 0.89, 2**	6.8/3.2 **9.2/4.2**
	Female, Lung A 2/50, 23/48, 28/48	0, 2,040, 4,052 ppm, 6 hour/day, 5 day/week	0, 63.7, 129.2 **0, 84.9, 170.4**	1.66, 0.2, 2 **1.57, 0.21, 2**	2.0/1.6 **2.6/2.1**
	Female, Lung C 1/50, 13/48, 29/48	0, 2,040, 4,052 ppm, 6 hour/day, 5 day/week	0, 63.7, 129.2 **0, 84.9, 170.4**	0, 1, 2 **0, 1, 2**	4.5/2.1 **6.0/2.8**

†Note:
Posterior median physiologically based pharmacokinetic (PBPK) values given in boldface type.
A = adenomas, C = carcinomas.
Chi-squared fit criterion is $p \geq 0.05$.

Table 19. Physiologically Based Pharmacokinetic (PBPK) Mouse Model Parameters for Dichloromethane†

Parameter	Prior Mean	Posterior Median
Cardiac Output L/hour	0.87	0.86
Tissue Blood Flows: L/hour		
GI Tract	0.1436	0.120
Liver	0.0148	0.0172
Fat	0.0409	0.0774
Poorly Perfused	0.240	0.249
Well Perfused	0.32	0.3096
Bone Marrow	0.077	0.086
Lung	0.0394	0.0442
Tissue Volumes: L		
GI Tract	8.75×10^{-4}	0.001
Liver	1.125×10^{-3}	1.25×10^{-3}
Fat	1.925×10^{-3}	1.75×10^{-3}
Poorly Perfused	0.0139	0.0135
Well Perfused	1.625×10^{-3}	1.75×10^{-3}
Bone Marrow	8.25×10^{-4}	1.0×10^{-3}
Lung	2.0×10^{-4}	2.5×10^{-4}
Partition Coefficients:		
Blood/Air	13.7	18.5
GI Tract/Blood	0.766	0.611
Liver/Blood	1.67	1.52
Fat/Blood	6.39	5.43
Poorly Perfused/Blood	0.618	0.654
Well Perfused/Blood	0.664	0.562
Bone Marrow/Blood	4.03	3.80
Lung/Blood	0.651	0.611

Table 19 (Continued). Physiologically Based Pharmacokinetic (PBPK) Mouse Model Parameters for Dichloromethane†

Parameter	Prior Mean	Posterior Median
Metabolic Parameters:		
Vmax, mol/hour	9.93×10^{-6}	9.51×10^{-6}
Km, *M*	1.59×10^{-5}	4.7×10^{-6}
KFC, $hour^{-1}(kg^{-1})^{-0.25}$	0.60	0.708
A1	0.405	0.28
A2	0.282	0.37
B1	0.271	0.25
B2	0.721	0.69

†Note:
Values are for a 0.025 kg mouse; V_{max} is the maximum metabolic rate of the liver mixed function oxidase (MFO), K_m is the concentration for half maximum reaction rate, K_{FC} is the first order rate constant for the cytosolic glutathione sulfotransferase (GST); A1 is the ratio of *in vitro* lung to liver (MFO) enzymatic activity normalized to microsomal protein; A2 is the ratio of lung to liver *in vitro* GST enzymatic activity normalized to cytosolic protein; B1 is the ratio of lung to liver tissue content of microsomal protein; B2 is the ratio of lung to liver tissue content of cytosolic protein. The prior mean parameters are the geometric mean mouse prior values from OSHA (1997) Table VI-5. The posterior median parameters are from OSHA's posterior probability distributions, Table VI-9.

Table 20. Linear Regressions of Pharmacokinetic Tissue Doses versus Applied Doses for Dichloromethane in the Mouse†

Study, Route, Site	Slope, b	Intercept, a	Correlation
Serota et al., drinking water ad lib., liver	0.61	-6.28	0.993
	0.35	**-15.1**	**0.916**
NTP, inhalation, lung	0.032	-0.445	0.999
	0.042	**-0.30**	**0.999**
NTP, inhalation, liver	0.0125	-1.03	0.997
	0.0153	**-3.08**	**0.985**

†Note:
Regressions are in the form (mg/kg-day) continuous physiologically based pharmacokinetic (PBPK) dose = a + b (applied dose). Inhalation applied doses are in ppm, oral applied doses are in mg/kg-day. Results based on posterior PBPK parameters are in boldface.

CALCULATION OF PHG

Noncarcinogenic Effects

Calculation of a public health-protective concentration (C, in mg/L) for DCM in drinking water for noncarcinogenic endpoints uses the following general equation adopted by U.S. EPA (1990, 1992a, 1996c):

$$C = \frac{NOAEL/LOAEL \times BW \times RSC}{UF \times DWC}$$

where

NOAEL/LOAEL	=	no observed adverse effect level or lowest observed adverse effect level,
BW	=	body weight (a default of 70 kg for a male or 60 kg for a female adult),
RSC	=	relative source contribution (a default of 20 percent to 80 percent as explained below),
UF	=	uncertainty factors (UFs) are included to account for gaps in our knowledge (uncertainty) about the toxicity of chemicals and for recognized variability in human responses to toxic chemicals.

In determining uncertainty factors for chronic effects it is conventional to apply an uncertainty factor (UF) where data are only available from short- or medium-term exposures of animals, rather than full lifetime exposures. In the case of DCM noncarcinogenic effects, there is an adequate chronic study in experimental animals of the critical effect of liver toxicity; the key study is of two-year duration or about the life span of a rat, so no uncertainty factor for study duration is necessary.

For interspecies extrapolation of toxic effects seen in experimental animals to what might occur in exposed humans an UF of up to 10-fold is generally recommended. This is usually considered as consisting of two 3.1-fold parts: one that accounts for metabolic or pharmacokinetic differences between the species; and another that addresses pharmacodynamic differences, i.e. differences between the response of human and animal tissues to the chemical exposure. Based on the limited metabolic studies of DCM in humans that indicate possible differences from rodents, and unresolved questions of its toxic potential for neurological, immunological and endocrine effects we believe a 10-fold UF for interspecies differences is appropriate.

Exposed humans are known to vary considerably in their response to toxic chemical and drug exposures due to age, disease states, and genetic makeup, particularly in genetic polymorphisms for enzymes (isozymes) for detoxifying chemicals. While little is known about individual variation of DCM metabolism and toxicity, the use of a 10-fold UF seems prudent considering the widespread use of tap water in the population.

Finally no additional UF is used to account for possible carcinogenicity. OEHHA has previously employed an additional 10-fold UF for other PHGs in situations where either a nonlinear dose response was applied to a carcinogen or where both linear and nonlinear approaches were used.

DWC = daily water consumption rate (a default of 2 L/day for an adult has usually been used by the U.S. EPA (1996b), or various L_{eq}/day to account for additional inhalation and dermal exposures from household use of drinking water as explained below).

A NOAEL of 6 mg/kg-day can be derived based on the NOAEL of 5.85 and 6.47 mg/kg-day for male and female rats, respectively, of the most sensitive noncarcinogenic effect in the liver from the two-year drinking water ingestion (Serota et al. 1986a) study. Using this NOAEL, the following calculation can be made:

$$C = \frac{6 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{100 \times 6 \text{ } L_{eq}\text{/day}} = 0.0133 \text{ mg/L} = 133 \text{ ppb}$$

In this calculation, a DWC value of 6 L_{eq}/day (a sum of 2.2 and 3.8 L_{eq}/day) is used to account for inhalation exposures via typical household use as well as ingestion of tap water. A 10-fold UF for interspecies differences with a 10-fold UF for individual variation of DCM metabolism and toxicity are used. The RSC of 20 percent is used to address other non-drinking-water sources, principally airborne DCM from volatile household substances. Support for these values is presented above in a discussion of exposure factors. This 133 ppb level is not considered to protect the public against the cancer effect.

Carcinogenic Effects

For carcinogens, the following general equation can be used to calculate the public health-protective concentration (C) for a chemical in drinking water (in mg/L):

$$C = \frac{BW \times R}{q_1^* \text{ or CSF} \times DWC} = \text{mg/L}$$

where

BW = adult body weight (a default of 70 kg),

R = *de minimis* level for lifetime excess individual cancer risk (a default for minimum individual extra lifetime cancer risk of 10^{-6}),

q_1^* or CSF = human cancer potency, or cancer slope factor. The q_1^* is the upper 95 percent confidence limit on the cancer potency slope calculated by the LMS model, and CSF is a potency derived from the lower 95 percent confidence limit on the 10 percent (0.1) tumor dose (LED_{10}). CSF = $0.1/LED_{10}$. Both potency estimates are converted to human equivalent [in $(\text{mg/kg-day})^{-1}$] using $BW^{3/4}$ scaling,

DWC = daily volume of water consumed by an adult (a default of 2 L/day or other volume in L_{eq}/day to account for additional inhalation and dermal exposures from household use of drinking water as explained above).

For the carcinogenic endpoint, the water concentration equivalent to a negligible lifetime theoretical cancer risk of 10^{-6} can be calculated as shown below. In the equation, the oral and inhalation CSFs of 4. 0×10^{-3} and 2. 4×10^{-3} $(mg/kg\text{-}day)^{-1}$ are derived using the linear approach based on the posterior mean parameters. The estimate of daily water ingestion is 2.2 L/day and 3.8 L_{eq}/day is the estimate of inhalation and dermal exposure equivalents from showering, bathing, flushing toilets and other household activities using DCM contaminated water (Bogen et al. 1992).

$$C = \frac{(70\ kg) \times 10^{-6}}{(4.0\times10^{-3} \times 2.2\ L/d) + (2.4\times10^{-3} \times 3.8\ L_{eq}/d)} = 3.9 \times 10^{-3}\ mg/L = 3.9\ ppb$$

For the prior mean parameters with oral and inhalation CSFs of 5.7×10^{-3} and 1.7×10^{-3} $(mg/kg\text{-}day)^{-1}$, the same calculation would be as follows:

$$C = \frac{(70\ kg) \times 10^{-6}}{(5.7\times10^{-3} \times 2.2\ L/d) + (1.7\times10^{-3} \times 3.8\ L_{eq}/d)} = 3.7 \times 10^{-3}\ mg/L = 3.7\ ppb$$

The average value is 3.8 ppb. Since the calculated public health-protective concentration based on noncancer toxicity of 133 ppb is less protective of public health than the above cancer based value of 3.8 ppb, the PHG level for DCM is therefore established at 4 ppb (rounded, 0.004 mg/L or 4.0 μg/L). This PHG value is considered to contain an adequate margin of safety for the potential noncarcinogenic adverse effects including adverse effects on the pulmonary, hepatic, renal, cardiac, hematopoietic, neurological, reproductive and developmental systems.

Table 21 lists the cancer slope factors described above together with a number of derived CSFs based on DCM equivalents and various internal to external conversions. In the first row of the table the CSF values ($0.1/LED_{10}$) are calculated using the continuous dose metric of mg/kg-day of GST metabolites generated by the liver and lung and scaled to human equivalent by ¾ power body weight. As noted above the PBPK dose metric is the mg GST metabolites/L liver or lung/day × L liver or lung/kg body weight. In the second row the same metrics are used to calculate the potency values using the LMS model for low dose extrapolation rather than the model-free extrapolation of $0.1/LED_{10}$. In the third row the slope values derived from the default method of applied dose without PBPK are given for comparison. In the fourth row the regression relations from Table 20 are used to derive external DCM equivalents. The regressions relate the continuous PBPK dose to the applied dose in mg/kg-day. The external DCM equivalents based on the inhalation regressions (Table 20) are adjusted for external human equivalent assuming 20 m^3/day of air inhaled and 70 kg body weight with complete absorption of inhaled DCM. In the fifth row the values in row four are adjusted assuming that only 75 percent of inhaled DCM is absorbed. In the sixth row external DCM equivalents are derived as in row five but using the q_1* as the initial slope factor. As can be seen the external values vary by less than a factor of two. As noted earlier the PBPK continuous dose metric based on the daily dose of GST metabolites in the liver and lung target tissues is considered the best measure of carcinogenic action in the mouse and, adjusted by ¾ power scaling, in humans.

Table 21. Human Cancer Slope Factors (CSF) and Calculated Public Health Protective Water Concentrations (C) Based on Mouse Liver and Lung Tumors†

Dose Metric	PBPK Para-meters	Oral Slope Factor {a} $(mg/kg\text{-}day)^{-1}$	Inhalation Slope Factor {b} $(mg/kg\text{-}day)^{-1}$	Inhalation Slope Factor {c} $(ppm)^{-1}$	InhalationSlope Factor {d} $(\mu g/m^3)^{-1}$	C {e}† (µg/L)
PBPK Continuous CSF [1]†	Prior	0.010	0.15	2.2×10^{-3}	6.3×10^{-7}	0.118
	Posterior	0.014	0.15	3.2×10^{-3}	9.2×10^{-7}	0.117
PBPK Continuous, LMS q_1* [2]†	Prior	0.012	0.17	3.0×10^{-3}	8.6×10^{-7}	0.104
	Posterior	0.016	0.17	3.4×10^{-3}	9.8×10^{-7}	0.103
DCM default method, applied doses [3]†	CSF	6.6×10^{-3}	2.3×10^{-3}	2.3×10^{-3}	6.6×10^{-7}	3.0
	q_1*	5.5×10^{-3}	2.5×10^{-3}	2.4×10^{-3}	7.0×10^{-7}	2.7
DCM external equivalent [4]†	Prior	6.1×10^{-3}	2.2×10^{-3}	2.2×10^{-3}	6.3×10^{-7}	3.2
	Posterior	5.6×10^{-3}	3.2×10^{-3}	3.2×10^{-3}	9.2×10^{-7}	2.8

Table 21 (Continued). Human Cancer Slope Factors (CSF) and Calculated Public Health Protective Water Concentrations (C) Based on Mouse Liver and Lung Tumors†

Dose Metric	PBPK Para-meters	Oral Slope Factor {a} $(mg/kg\text{-}day)^{-1}$	Inhalation Slope Factor {b} $(mg/kg\text{-}day)^{-1}$	Inhalation Slope Factor {c} $(ppm)^{-1}$	InhalationSlope Factor {d} $(mg/m^3)^{-1}$	C {e}† (mg/L)
DCM external equivalent, 75 percent absorption [5]†	Prior	5.7×10^{-3}	1.7×10^{-3}	1.6×10^{-3}	4.7×10^{-7}	3.7
	Posterior	4.0×10^{-3}	2.4×10^{-3}	2.4×10^{-3}	6.9×10^{-7}	3.9
DCM external equivalent q_1* based, 75 percent absorption [6]	Prior	5.5×10^{-3}	2.25×10^{-3}	2.24×10^{-3}	6.43×10^{-7}	**3.4**
	Posterior	4.2×10^{-3}	2.55×10^{-3}	2.54×10^{-3}	7.29×10^{-7}	3.7

†Notes:

[1] PBPK continuous dose in mg/kg-day of GST metabolites generated by the liver and lung scaled to human equivalent by ¾ power body weight. CSF = $0.1/LED_{10}$ (human). LED_{10} is the lower 95 percent confidence bound on the dose associated with a 10 percent increased tumor incidence.

{a} Column a is the oral slope factor determined from GST metabolites generated by liver ¾-power scaled to human equivalent in $(mg/kg\text{-}day)^{-1}$.

{b} Column b is the inhalation slope factor determined by the GST metabolites generated by liver and lung 3/4-power scaled to human equivalent in $(mg/kg\text{-}day)^{-1}$.

{c} Column c is the inhalation slope factor from {b} converted to $(ppm)^{-1}$ using the regressions from Table 20 that were forced through zero. Each site was calculated separately, sex averaged, then summed, e.g., the lung prior sex averaged CSF is 0.023 $(mg/kg\text{-}day)^{-1}$. Since 1/0.023 = 43.48 mg/kg-day and 48.43/0.032 = 1,359 ppm, 1/1,359 ppm = 7.4×10^{-4} $(ppm)^{-1}$. Adding this value to the prior liver value of 1.41×10^{-3} $(ppm)^{-1}$ gives 2.15×10^{-3} $(ppm)^{-1}$. This appears as 2.2×10^{-3} $(ppm)^{-1}$ above.

{d} Column d is derived from {c} using the conversion factor of 0.287 ppm/(mg/m^3).

{e} Column e, the health protective concentration C = 70 kg $\times$ 10^{-3}/[(oral CSF $\times$ 2.2 L/day) + (inhalation CSF $\times$ 3.8 L_{eq}/day)] = µg/L.

[2] Same dose metric as [1] but q_1* based on linearized multistage (LMS) model.

[3] Default methodology approach based on applied doses i.e., no PBPK.

[4] External DCM equivalents based on oral and inhalation regressions of PBPK dose versus applied dose (Table 20) and adjustment for inhalation (column c) as follows: $(ppm)^{-1} \times 0.287$ ppm/$(mg/m^3) \times$ one day/20 $m^3 \times 70$ kg = $(mg/kg\text{-}day)^{-1}$.

[5] As above but assumes 75 percent low dose absorption of DCM in humans (Bogen et al. 1992).

[6] As above but external DCM values derived from the q_1* values in [2] rather than the CSF in [1].

In conclusion, **the PHG for DCM is established as 0.004 mg/L or 4 ppb based on liver and lung cancer in mice exposed by either the oral route in drinking water or by inhalation.** In

developing the PHG, a PBPK model was used for assessing target tissue metabolism following oral or inhalation exposures similar to exposures that resulted in mouse liver and lung tumors. The dose metric used was mg GST metabolites generated by liver and lung per kg body weight per day converted to external DCM equivalents.

RISK CHARACTERIZATION

Noncarcinogenic Effects

Noncarcinogenic health effects are not expected to result from the generally low exposures to DCM in drinking water.

Carcinogenic Effects

Inhalation exposure to DCM produced increased incidences of liver and lung tumors in both male and female mice, salivary gland sarcoma in male rats, leukemia in female rats, and mammary tumors in both sexes of two strains of rats. Oral exposure to DCM in drinking water produced liver tumors in female rats and male mice. Exposure to DCM through gavage or intraperitoneal injection provides further evidence for the carcinogenicity.

- This assessment of the cancer risk of DCM exposure in human populations via DCM-contaminated drinking water depends on the assumption that DCM metabolism via the GST pathway is the critical event in humans as well as in experimental animals. Alternatively the MFO pathway may play a significant role in DCM carcinogenicity in humans. In addition if the sensitivity of humans to the carcinogenic action of DCM is not reflected by the most sensitive site seen in animals (mouse liver tumors) adjusted by ¾ power scaling, this will also affect the accuracy of the assessment. At this time it is impossible to accurately evaluate these alternative possibilities.
- The assumption of the key role of GST metabolites in DCM carcinogenesis is based on several lines of independent evidence.
    - The dose dependency of DCM-induced lung and liver tumors correlates with the lack of saturation kinetics expected of the GST pathway.
    - The relative species tumor sensitivity correlates with measured GST activity, i.e., mice > rats > hamsters.
    - There is a dependence on glutathione for mutagenicity of DCM in the Ames *Salmonella typhimurium* test.
    - Increased mutagenicity is seen in bacteria transfected with multiple copies of 5,5-GST.
    - The cellular localization of 5,5-GST in the mouse nucleus correlates with the higher sensitivity of the mouse. No nuclear localization is seen with MFO enzymes.
    - Two DCM metabolites of the GST pathway are capable of direct interaction with DNA.
    - DNA damage in Chinese hamster ovary cells induced by DCM requires the presence of glutathione.
- GST activity with DCM has been measured in human volunteers (ca. 50).

- *In vivo* pharmacokinetic studies in human volunteers were accurately predicted by a PBPK model based on *in vitro* studies in rodent and human liver and lung samples.
- DCM has not been convincingly demonstrated to cause cancer in humans exposed in occupational settings. OEHHA concludes that the available human occupational epidemiology studies are of little value in human risk assessment, e.g., in setting upper bounds on human DCM carcinogen slope factors.
- The dose metric of choice in evaluating the mouse carcinogenicity data is the flux of GST pathway metabolism in the target tissues determined by PBPK analysis expressed on a continuous mg per kg body weight per day basis and converted to external DCM equivalents. Since DCM, the parent compound, is not reactive with DNA itself, various dose metrics based on DCM such as peak blood concentration, area under the blood concentration times time curve (AUC), target tissue AUC, and total metabolized dose may be of limited use in assessing DCM cancer risk. While the use of this PBPK metric carries uncertainties it is considered the metric most closely associated with the carcinogenic effects observed in the experimental animals.
- The human PBPK model (OSHA 1997) with typical DCM exposure of 10 μg/day ingestion (5 μg/L $\times$ 2 L/day) plus 20 μg/day by inhalation (4 Leq/day) predicted negligible extra lifetime cancer risk (8×10^{-7}) based on a daily generation of GST metabolites in liver and lung. Since the PBPK model was only a single 24-hour simulation, longer simulations allowing steady state concentrations of GST metabolites would give a slightly higher risk prediction. The use of a human model assumes interspecies tumor site and pharmacodynamic concordance, which are still uncertain for DCM. The results of the human model estimate is consistent with the PHG of 4 ppb or 4 μg/L notwithstanding the limitations noted above.
- The risk values predicted by this assessment are comparable with those of the OSHA (1997) assessment for occupational airborne exposures when the latter are adjusted for lifetime community ambient air exposures, i.e., 0.94×10^{-3} $(ppm)^{-1}$ air (OSHA) versus 1.65×10^{-3} $(ppm)^{-1}$ air (OEHHA). The small difference may be due to methodological differences since OSHA used the MLE of extra risk for the 95th percentile of the GST distribution of metabolites whereas OEHHA used the central estimates of GST metabolites generated but the 95 percent lower bound on the GST metabolite dose yielding a 10 percent tumor response (i.e., LED_{10}).
- The use of a lifetime average daily dose does not consider possible dose rate effects or the impact of less than lifetime exposure to DCM. Also it is unknown whether children are at greater risk from DCM exposures than are other segments of the population.

The exposure parameters used for DCM in this analysis of 6 L_{eq}/day are typical for volatile organic chemicals. The CalTOX™ environmental fate program predicts a total relative household exposure via DCM contaminated groundwater of 5.8 L_{eq}/day.

OTHER REGULATORY STANDARDS

California ARB has listed DCM as a TAC (Health and Safety Code Section 39655) since 1989 under Assembly Bill (AB) 1807 of 1983, and as a chemical to be reported under the Air Toxics "Hot Spots" (AB 2588 of 1987) Program (Health and Safety Code Section 44300). On April 27, 2000, the ARB approved a regulation that would require that brake cleaners, carburetor

cleaners, engine degreasers, and general purpose degreasers manufactured for sale in California not contain DCM. ARB also made changes that would expedite the effective dates of the

regulation to reduce emissions of chlorinated TACs including DCM from automotive maintenance and repair activities shown on http://www.arb.ca.gov/regact/amr/amr.htm. OEHHA has listed DCM as a carcinogen under Proposition 65 of 1986 (Health and Safety Code Section 25249.5).

U.S. EPA Maximum Contaminant Level (MCL)

On July 17, 1992, U.S. EPA (1992b) promulgated an MCL of 5 ppb for DCM (40 CFR 31789), basing their assessment on the increased incidence of hepatocellular adenomas and carcinomas in the NTP (1986) inhalation study and the increased combined incidences of hepatocellular adenomas and carcinomas in the Serota et al. (1986b) drinking water ingestion study using the U.S. EPA (1986) Guidelines for Carcinogen Risk Assessment. The oral cancer slope factor of 7.5×10^{-3} (mg/kg-day)$^{-1}$ used to derive the MCL of 5 ppb is an arithmetic mean of the slope factors from these two studies, the inhalation slope factor from female mice $q_1^* = 2.6 \times 10^{-3}$ (mg/kg-day)$^{-1}$, and the oral slope factor from male mice $q_1^* = 1.2 \times 10^{-2}$ (mg/kg-day)$^{-1}$.

The inhalation (NTP 1986) study was used by the U.S. EPA (1992b) together with the oral (Serota et al. 1986a, 1986b) studies to derive the drinking water standard for DCM because DCM is absorbed rapidly following either inhalation or oral exposure and the elimination kinetics of DCM were consistent with results generated by both oral or inhalation exposures. Although U.S. EPA (1992b) has assessed the inhalation pharmacokinetic model to estimate the risks of ingestion, this information or metabolic data has not been used to modify the mean oral slope factor of $q_1^* = 7.5 \times 10^{-3}$ (mg/kg-day)$^{-1}$ used to calculate the MCL. Based on this potency estimate, a 10^{-6} lifetime cancer risk of 5 ppb was derived as the MCL for DCM using a body weight of 70 kg and a DWC of 2 L/day.

If the U.S. EPA's mean slope factor from the Serota et al. (1986b) studies adjusting for uptake and metabolism of DCM is used, a lower number of 1.6 ppb is obtained. If the slope factor of 7.5×10^{-3} (mg/kg-day)$^{-1}$ derived by the U.S. EPA (1992b) is applied to calculate the 10^{-6} risk level using a body weight of 70 kg and a DWC of 6 L_{eq}/day, a lower number of 1.6 ppb is obtained, compared to the MCL of 5 ppb. On the other hand, the analysis by DHS (1989) included corrections for DCM uptake and metabolism to generate an oral cancer slope factor of $q_1^* = 3.5 \times 10^{-3}$ (mg/kg-day)$^{-1}$. If this cancer slope factor of 3.5×10^{-3} (mg/kg-day)$^{-1}$ is used to calculate the 10^{-6} risk level using a body weight of 70 kg and a DWC of 6 L_{eq}/day, a lower number of 3 ppb or μg/L is obtained. This 3 ppb level was proposed by OEHHA as a RPHL in 1993 but was not finalized.

Other drinking Water Standards Standards and Guidelines

The States of New Jersey and Rhode Island established drinking water standards for DCM of 2 ppb. The guideline for DCM in drinking water is 4.7 ppb in Arizona, 25 ppb in Connecticut, 50 ppb in Kansas, 48 ppb in Maine, 5 ppb in Massachusetts, 50 ppb in Minnesota, 5 ppb in New Hampshire, and 5 ppb in Vermont, respectively (ATSDR 1993, HSDB 1999, Sittig 1994).

In Canada, the Maximum Acceptable Concentration (MAC) established by Health and Welfare Canada (HWC) in 1989 for DCM in drinking water is 50 ppb under the Guidelines for Canadian Drinking Water Quality; this level is also used as the Interim Livestock Water Supplies Guidelines for DCM (HWC 1992). Canada also adopted the recommendations of the U.S. EPA as the Water Quality Objectives for Quebec (ATSDR 1993, Environment Canada 1993, OECD 1994). The Japan Environmental Quality standard for DCM in water was 20 ppb in 1993. In

Austria, the sum concentrations of 14 different halogenated aliphatic hydrocarbons including DCM must not exceed 30 ppb in drinking water. In Denmark, the DCM limit in domestic drinking water is 20 ppb. In the Netherlands, the target limit for DCM is 0.5 ppb in surface water and 0.2 ppb in groundwater (OECD 1994).

Other Standards and Guidelines

OSHA regulates DCM under the Hazard Communication Standard and as a chemical hazard in laboratories. The OSHA (1997) has established an occupational exposure limit (TLV-TWA) of 25 ppm for an eight-hour workday with an action level of 12.5 ppm, and a short-term exposure limit (TWA) of 125 ppm for 15-minute durations for persons who work with DCM. NIOSH recommends a permissible limit of 75 ppm of DCM over a 10-hour workday in the presence of CO concentrations less than or equal to 9.9 ppm.

As early as 1946, ACGIH recommended an occupational exposure limit Threshold Limit Value (TLV) of 500 ppm for DCM. In 1975, ACGIH lowered the recommendation to 100 ppm. NIOSH recommended a reduction to 75 ppm as an eight-hour TWA in 1976. In 1988, ACGIH lowered the TLV to 50 ppm as an eight-hour TWA. NIOSH issued Questions and Answers on DCM risk and control in furniture stripping process in 1993.

CPSC regulates household products containing DCM, considering them to be hazardous and subjecting them to labeling requirements since 1985. The FDA has established limits for residual DCM in food since 1967; the amount that can remain in spices is 30 ppm, in hops extract 2.2 percent or 5 ppm, and in decaffeinated coffee 10 ppm. FDA proposed in 1985 and finally banned the use of DCM as an ingredient in aerosol cosmetic products in 1989.

The U.S. EPA regulates DCM under the Clean Air Act (CAA), Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Clean Water Act (CWA), Food, Drug, and Cosmetic Act (FDCA), Resource Conservation and Recovery Act (RCRA), Superfund Amendments and Reauthorization Act (SARA), Toxic Substances Control Act (TSCA) and Safe Drinking Water Act (SDWA) (NTP 1998). U.S. EPA (1993) characterized DCM as the third most commonly used halogenated solvent based on 1991 data. U.S. EPA requires that releases of DCM of 1,000 pounds or more be reported to the federal government. DCM is one of the Hazardous Air Pollutants (HAPs) under the 1990 Amendments of CAA. DCM is a priority pollutant under the amended CWA and was characterized as one of the most significant priority pollutants to be addressed (U.S. EPA 1997). U.S. EPA (1995) is proposing effluent limitation guidelines for the pharmaceutical industry. The U.S. EPA Health Advisory recommends that exposure of children to DCM in drinking water should not exceed 10 ppm for one day, or 2 ppm for 10 days.

DCM is listed as a priority substance under the Canadian Environmental Protection Act and has been classified as a toxic substance under Canadian legislation dealing with occupational health and safety, foods, transportation, water, and consumer protection (Environment Canada 1993, OECD 1994). Canadian Interim Water Quality Guidelines recommended 98 ppb as the level for the protection of freshwater aquatic life. Canada also adopted the maximum residue level of DCM as a food additive established by the U.S. FDA.

The Japan Society for Occupational Health (JSOH 1996) recommends Occupational Exposure Limits. Both the Japan Ministry of Labour and the Japan Environmental Agency regulate DCM (OECD 1994).

DCM is classified as harmful and as a carcinogen in the third category by the Commission of the European Union according to the Annex to Commission Directive 93/72/EEC of September 1, 1993. Most of the European countries, such as Austria, Australia, Belgium, France, Germany, Switzerland, and United Kingdom, adopt the TLV recommended by the ACGIH of the U.S. (OECD 1994).

The Health and Safety Commission (HSC) of the United Kingdom in 1994 established two Maximum Exposure Limits (MEL) under the Control of Substances Hazardous to Health (COSHH) Regulations based on acute CNS effects, a short-term limit of 300 ppm and an eight-hour TWA limit of 100 ppm (HSC 1994). The United Kingdom Woodworking National Interest Group issued Health and Safety Executive in 1992 on DCM health risks during furniture stripping (Gardner 1994).

German Ordinance on Hazardous Substances classifies DCM as "suspected or being carcinogenic" and considers DCM dangerous to the environment. A biological workplace tolerance value for DCM of 100 μg/dL as defined by the COHb level in blood was set by the Germany government (OECD 1994). The German MAK Commission for the investigation of health hazards of chemicals in the work area established 100 ppm or 360 mg/m^3 of DCM as the maximum concentration in the workplace for 8 hours per day for 40 hours per week (Sullivan et al. 1993).

The French Labor Ministry considered DCM an occupational carcinogen. The Dutch Expert Committee on Occupational Standards (DECOS 1993) evaluated toxicological data to establish a health-based recommended Occupational Exposure Limit (OEL) for DCM. Derivation of an OEL for DCM based on acute CNS effects and relative potency analysis was proposed by Storm and Rozman (1998). The Swiss Law for Toxic Substances classifies DCM in Poison Class 4 by virtue of its acute toxicity. In the Netherlands, the target limit for DCM is 20 μg/m^3 in air and 0.05 μg/kg in soil (OECD 1994).

The Austria Maximal Workplace Concentration classifies DCM as potentially carcinogenic, and the Chemical Ordinance (208/89) classifies DCM as harmful with possible risk of irreversible effect. Austria requires compulsory medical supervision of workers exposed to DCM and prohibits the use of DCM by young workers and expectant mothers. Austria lowered the worker exposure standard for DCM from 100 to 50 ppm TWA in 1991 (OECD 1994).

The Danish Ministry of Labor has listed DCM as a carcinogenic substance since 1988 and the OEL is 175 mg/m^3. Danish Ministry of Environment classified DCM as a third category carcinogen. DCM was identified as one of the 18 "risk chemicals" of neurotoxicity to which workers were exposed in the 25 most hazardous industries in Denmark (Simonsen and Lund 1992). The government of Finland has classified DCM as a carcinogen in the third category. The Norway National Pollution Control Authorities and the Directorate of Labour Inspection's Scientific Group for Identification of Carcinogens has classified DCM as harmful by inhalation and as a low-potency carcinogen. The occupational exposure limit in Norway was 35 ppm in 1994 (OECD 1994).

The Swedish National Chemicals Inspectorate has classified DCM as a low-potency carcinogen and the Institute for Environmental Medicine at Karolinska Institute has specified a low risk level in ambient air with reference to the carcinogenic effect. In 1991 Sweden began measures to phase out the marketing and use of DCM (OECD 1994). In 1990, DCM was classified as a carcinogen by the National Institute of Occupational Health in Sweden, and the Swedish eight-hour PEL is 35 ppm. DCM is banned from consumer products in Sweden as of 1993 and from products for all professional use as of 1996 (Anundi et al. 1993, OECD 1994).

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